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MILTON C. WINTERNITZ NUMBER

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MILTON C. WINTERNITZ

From a crayon portrait by Deane Keller

MILTON C. WINTERNITZ

It is eminently appropriate that the Editors of the Yale Journal of Biology and Medicine should issue a special number in honor of Dr. Winternitz, prepared by his associates, colleagues, and former students. There could be no more fitting expression of the sense of gratitude which Yale cherishes for the service which Dr. Winternitz has rendered to the University. His career here has manifested the rare combination of the imaginative administrator, the stimulating teacher, and the tireless scientific scholar.

The Yale Medical School of today is the result of his fifteen-year Deanship. There is no period of more brilliant development in the history of Yale. Returning to his laboratory he achieved the miracle, so rare in the career of an academic administrator, of successful resumption of scientific scholarship. It was inevitable that he should be called into vitally important national service as the country faced the crisis of war. Yale is proud of the range and quality of that service as she is of the prestige which his name has brought to our faculty.

As Dr. Winternitz retires from the Anthony M. Brady Professorship of Pathology, Yale salutes him and expresses her vivid sense of admiration and affection.

CHARLES SEYMOUR

DR. WINTERNITZ AS DEAN OF THE SCHOOL OF MEDICINE*

JAMES ROWLAND ANGELL

After fifteen years of brilliant service as Dean of the School of Medicine, Dr. Milton C. Winternitz retires and will give his entire time to his professorship in pathology. . . . Dr. Winternitz' achievement has few parallels in American educational history. When he took charge, the School of Medicine faced the most disheartening prospects. The faculty had just lost by resignation a number of its ablest men at the head of important clinics. The plant was scattered, cramped, outworn, and outmoded. The hospital facilities were also distressingly inadequate, both in quantity and in quality, for the purposes of a modern school. The students were few in number and in many cases unsatisfactory in ability and personality. The alumni of the University were many of them hostile to the effort to build up a first-class medical school at Yale, among them not a few important physicians. The University community was anything but sympathetic. The school and the hospital were both crippled by lack of funds. Altogether the outlook was as unpromising and depressing as could well be conceived.

Into this situation was injected the dynamic personality of the new Dean. He brought a dauntless spirit which could not be discouraged, extraordinary imagination, sound ideals, and a capacity for endless hard work. His enthusiasm was contagious, and, while the story cannot be here fully related, the result is a medical school everywhere recognized as among the leaders in the United States, a greatly enlarged faculty of outstanding scientific and medical experience, a carefully chosen student body restricted in number to the teaching and laboratory facilities of the school, an adequate plant, including laboratories and hospitals, of the most modern kind, with few equals anywhere in the world, and greatly enlarged endowments.

These successes are in part due to the sheer drive of the Dean and the support of his staff, but they are in no small measure attributable to the ideas which he has promoted. He has not only stressed in season and out the preventive aspect of medicine as its great obligation. This others have done, thought none more ably. But he has also emphasized the concept of

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* Reprinted from the *Report of the President for the Academic Year 1934-1935.* Mr. Angell (1869-1949) was associated with Dr. Winternitz in many phases of the reorganization of the School of Medicine.

positive health as the dominating ideal to which medicine must now devote itself, teaching men how to live sanely and vigorously and not merely how to forestall disease or how to cure it when it appears, important as are both these ends.

As a by-product of this principle, he has constantly dwelt upon the responsibility of medicine to concern itself with the whole man and not simply with his diseased organs, to take into full and intelligent account in caring for him his entire social and economic environment. This position can be lampooned, if taken unfairly out of context. A man with a sliver in his finger wants quick and simple surgical help and, once rendered, that is likely to be the end of the episode. To bring remote economic and social considerations into the picture would be silly. But in serious, continuing illness, and still more in the preventive activities of medicine and in the development of the program of positive health, these considerations are not only not ridiculous, they are absolutely indispensable.

Many of these ideals, together with the conception of the coördination of all the available sciences and technical skills to meet the basic and enduring problems of man in society, are perhaps most graphically embodied in the Institute of Human Relations, in whose founding Dr. Winternitz was a leading figure.

He has also contributed to many improvements in the methods of teaching medicine to students. Very small classes, highly individualized instruction, and the stimulation of the student's own initiative and responsibility for his development—all have received his energetic encouragement. Not the least important accomplishment has been the development of a strong library, which is now an integral part of the life of the school. . . .

The University is under deep and lasting obligation to Dr. Winternitz, and the School of Medicine will always stand as a monument to the wise and devoted service which he rendered at a critical period.

PREFRONTAL LOBES AND SOCIAL DEVELOPMENT*

SPAFFORD ACKERLY

Writing this article for the issue of the Journal honoring Dean Winternitz has given me great joy and deep satisfaction. It embodies, I hope, something of the scientific spirit to which I was exposed at Yale under his able and inspiring leadership.

The use of prefrontal lobotomy in the amelioration of the effects of certain forms of mental illness in the last decade and a half has given the clinician and investigator much food for thought not only in regard to the meaning of the changes observed as a result of this operation, but also in comparing new data with other brain deficit studies in man and animal. The thesis presented here on the role of the prefrontal lobes in the social development of man is occasioned by clinical observation of the behavior of persons who have suffered known brain damage, more particularly located in the prefrontal areas. It is of medical interest to know how these individuals behave as persons, especially as personalities living with other personalities, in short, as surviving bio-social organisms. Furthermore, as new or additional data turn up it is of scientific interest to re-examine some aspects of old concepts of mind-brain functioning and of so-called higher and lower orders of thinking, feeling, and action as related to new and old brain structures, as well as the more recent ones of mass action and over-all milieu participation, the unitary concept early accented in medicine by Jackson[11] and later developed by others, particularly Goldstein.[7] The evidence presented here I think should help to answer the question as to whether evolution is concerned as much with developing hierarchies of functioning as it is with the development of the wherewithal to cope ever more finely and effectively with an ever-changing world and with other similar organisms similarly developing, and whether this wherewithal is dependent particularly on the intactness of the prefrontal lobes.

It is postulated that especially in the forebrain reside the possibilities of developing and elaborating social and antisocial concepts, attitudes, and drives, and that without the forebrain man is predominantly an asocial

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being. Into the fine coping mechanisms of a social nature such as professional people are called upon to use in daily contacts with their fellowmen go, of course, all those intellectual functions that are being tested quantitatively today and many that cannot be tested. Therefore, it would be well first to touch on the work of a few authorities who have been testing the more specific intellectual functions in animals and man with bilateral prefrontal lobe cortical and subcortical deficit, before proceeding to consider the test findings in the fibre-damaged leukotomy cases. After that we shall consider the clinical aspects of both types of cases, their probable meaning and significance.

I have the generous permission to quote the following from a personal communication from Dr. K. S. Lashley:[19]

Recent studies at the Yerkes Laboratories, chiefly by Drs. Karl Pribram, Robert Blum, Josephine Blum, and K. L. Chow, are changing our conceptions both of the permanence and of the specificity of symptoms from prefrontal lobe damage. The usual syndrome of hyperactivity and loss of capacity for delayed reaction and allied functions has been confirmed with rhesus monkeys. However, the changes in activity are variable with apparently similar lesions. The capacity for delayed reaction improves markedly with continued post-operative training. The capacity for delayed reactions is also lost after temporal lobectomy and the function is disturbed after extensive lesions in the parietal and prestriate regions. Topectomies have produced relatively slight disturbances, maximal after destruction of the middle frontal gyrus, less from the superior, and little or no effect from removal of the inferior or orbital gyri.

Chimpanzees give a different picture. Two animals, now about three years old, had prefrontal lobectomies at about six weeks. They have been at all times within the range of normal controls in their physical and behavioral development. Two adults with prefrontal lobectomy and one with orbital gyri removed have shown no demonstrable symptoms. There is no restless pacing and, although there is a suspicion of increased activity when they are in the large compound, the difference is unreliable. There has been no loss of capacity for delayed reaction. They have learned delayed matching in normal time. There has been no change in emotionality, including reaction to frustration. These animals have not yet been sacrificed and anatomic controls are lacking but I am confident that the lesions extend to the caudal limits of the granular cortex.

The difference between the monkeys and chimpanzees is difficult to explain. It has been suggested that the chimpanzee has alternative ways of doing the delayed response (some representative symbolism) equivalent to a language function which dispense with a more kinesthetic frontal lobe action but there is no evidence to support this.

In general the experimental work with animals has contributed little if anything to understanding of the functions of the prefrontal granular cortex. Harlow and I have suggested independently that the delayed reaction involves a conflict of tendencies to response or conflict of "sets." This view relates the loss from lobectomy to the human confusions in categorizing that have been emphasized by Goldstein, but it does not

help to understand either the difference between the chimpanzee and monkey or the production of the same symptom by lesions in the posterior association areas.

Particularly interesting is the account of the two chimpanzees who, compared with controls, show no signs of impairment or change in "emotionality" even under frustration and yet had frontal lobectomies at the early age of six weeks.

Later on will be discussed the clinical histories of a human male who at 19 years of age was discovered to have extensive bilateral frontal lobe deficit, congenital in origin,[8] and Spurling's case[3, 15] of a 32-year-old woman with less than one quarter of all prefrontal lobe tissue remaining after a tumor operation. On tests, however, both of these cases showed what Lashley and Harlow described independently as conflicts of "sets." They both resist shift in conversation and become irritable when interrupted in what they are doing. Both cases were referred to Halstead for study and are in his series of 237 subjects and controls to whom he gave his four factor analysis tests as a measure of "biological intelligence."[9] Both showed definite impairment especially of the A factor of abstraction and the cerebral power factor P. Dr. Halstead[10] has the following to say about this congenital case, unique in medical literature so far as prolonged clinical study and measurements are concerned and perhaps unique in the extent and location of his pathology and subsequent development.

Laboratory studies on J.P. enable us to evaluate him in the light of our quantitative and qualitative studies made on a wide range of neurological and psychiatric patients. From a quantitative standpoint, one of the most conspicuous features of his test behavior was his inferior performance on our Category Test, a measure of the A factor. This, coupled with his low cerebral power factor P, reveals an underlying deficit in the ability to sustain processes of rational abstraction long enough to reach the stage of good judgment. He is thus lacking in essential mechanisms for healthy growth and differentiation of ego functions. This deficit is evidenced in his social adjustments by failure to learn, either positively or negatively, from his social experiences. He thus seems to have gotten neither better nor worse in his total adjustments. He becomes neither more committed to the "good" life than to the "bad." He quite literally fails to "profit from experience." It is believed that his frontal lobe deficits deprive him of important feed-back from his responses. . . . This becomes apparent more in his adjustment to new situations requiring abstraction than in his habit determined adjustments in simple face-to-face social situations. He is in many ways a creature of whimsies, but, for the most part, his whimsies do not appear to be dangerous or anti-social. They are more likely to be asocial. He is thus a shell of a human being, capable of superficial manifestations of poise, simple social graces, alertness, and verbal fluency.

In 1933 Genette Ulvin Burress[4] found this patient to have average

intelligence but his performance on the Porteus Maze Test, a test specifically designed to assess planning ability, was significantly below the level of the other performances. Dr. Catherine Miles[13] in 1936 summarized her tests on this patient as follows: "Were this patient able to reason abstractly relatively as well as he is able to deal with materials in view, his intelligence would rate superior. The organic disability seems to be definitely registered in this contrast." Dr. Horsley Gantt[6] studied this patient in 1936 and found some impairment of the function of adaptability as measured by a simple conditioned reflex test.

In Brickner's case[3] intelligence tests were inconclusive, yet all his reactions seemed very immature. In summary Brickner states: "The emotional side of A's conduct rests fundamentally upon an intellectual deficit gained from the record not the tests, namely, the inability to synthesize simple thought processes into more complex structures. Thus are produced disturbances depending upon the separate activations of relatively simple units which formerly were synthesized. There is nothing," he continues, "in A's record to indicate primary participation of the frontal lobes in emotional function or that emotional function as such is primarily disturbed by the destruction of the frontal lobes. Concerning sex failure in A it is inescapable that in A the advance to an adult sex level preoperatively was accomplished at least in part by virtue of function in which the frontal lobes were concerned." "Only once is he known to have even attempted to strike anyone." It seems to me that these facts and others compiled since then point to very important participation of the frontal lobes in emotional function including the sex drive.

So much for measurements on selected cases showing extensive cortical and subcortical destruction of both frontal lobes. Let us now consider test results on frontal leukotomy cases. In the Greystone-Columbia project[5] no quantitative evidence of intellectual impairment was found in their carefully tested and controlled series. In summary they state: "No existing theory or hypothesis dealing with the psychologic significance of the human frontal lobes is tenable. There is no point in either propping up or demolishing established theories. They add only to the confusion of thought and had best be forgotten. With the body of evidence now available from this study there is no clear outline of a useful and unifying formulation which would readily replace the older theories. We believe that the way is clear for a comprehensive investigation which should lead to new knowledge which

will be meaningful." Neither did Halstead[9] find any changes in his frontal leukotomy cases. I accept their negative findings, but object to the Columbia group's conclusions.

These negative findings and the relatively few changes measured even after lobectomy operations on man and animal serve well to highlight the marked changes that have been noted clinically in the social and emotional life of patients with extensive frontal defect, showing how small a segment of man's total behavior is being quantitatively assessed today.

I should like now to summarize briefly the reactions of four of my patients to illustrate not only the marked changes that have taken place in their intellectual, social, and emotional lives, but also to point up the question as to why their drives and impulses are not more dynamic and forceful than ever, released as it seems they should be from cerebral control and restraint. It is practice and not theory alone that must answer such questions as the one raised by an intelligent, distraught patient, frightfully uncontrolled in each of several psychotic attacks, "If I am already being torn asunder, why on earth operate on my brain and take away what controls I have left?"

The two cases with severe cortical and subcortical damage mentioned above will be discussed first (remembering that the brain defect in one dates from infancy), followed by a brief history of the two leukotomy patients.

For purposes of brevity I shall mention some of the main characteristics that are more or less common to all four patients, particularizing later. All are non-self-supporting and all show childlike unconcerned acceptance of the dependency role as though not concerned with personal security, comfort, or other personal ends. There is no clinging to others for financial or emotional support. They seem curiously detached in this respect. They want to contribute to others and do so frequently but for the most part only with little gifts at their disposal and with little helpful acts. In general, they all show simple, monotonously sustained amiability punctuated at times with displeasure and irritability upon sudden interference with their activities of the moment; defective motivation for any sustained complicated behavior and whimsical in every-day affairs; defective planning ability outside of immediate goals. In these simple immediate goals, however, they have apparently never set out to do anything unworthy, destructive, or antisocial. On the other hand, none is a contributing social being in the mature sense.

All are more or less cut off from sustained interpersonal relationships. However, all have what seems to be simple, sincere, childlike social feeling. The cortical case operated on for a tumor at 37 showed postoperatively the highest degree of initiative, planning power, and social adjustment of the four. Her preoperative general adjustment was normal and mature. There is more euphoria, energy, alertness, concreteness, one-trackness, and emotional lability in the cortical cases than in the two subcortical. Both of the latter had made poor pre-illness personality adjustments from earliest days, one especially so. This undoubtedly has markedly influenced the post-operative course. There is evidence of carry-over of other pre-illness characteristics such as extraordinarily good insight in one leukotomy case. She always had remarkable insight and still shows a surprising amount in direct contrast to the other who has always shown a profound lack of it. Since the one whose insight is still somewhat preserved was operated on only eight months ago, it is difficult to know what use she will make of it as time goes on.

Moreover, in thinking of these four people, one is struck particularly with the absence of all compensatory drive when there exist in man such rich varieties of it, running the whole gamut of patterns from the most refined social behavior to the most crude, deadly, and dangerous. The hobo is free to choose to read philosophical books, to live in his inner world determined to be thus free, and willing to pay the price, coming out only in search of food and shelter; but these patients are not free—they are more stimulus bound, content to live in the here and now.

The first case, already reported elsewhere,[1] is a 52-year-old Hungarian woman, wife of a coal miner; mother of four children; uneducated, average intelligence, a thrifty, capable, loving wife, mother, neighbor, and friend; happy, outgoing, fun-loving, generous, active, religious; well thought of and respected in the little mining community. She was operated on in 1933 at 37 years of age for a large meningioma arising from the cribriform plate. After the three-stage operation by Spurling, the only prefrontal lobe tissue remaining on either side was "the tip of the left prefrontal lobe [which] was no more than 1 cm. in thickness and literally floated in the bath of Ringer's solution." On admission to the hospital she was practically moribund, having suffered head pains for several years with increasing severity. In spite of a markedly elevated mood the first several months following operation, she showed a surprising "persistency and deliberation in speech and action and lack of distractibility." As to the happy mood, facetiousness, and childish behavior, and her heightened sex interests at that time she said, "I was dead from my suffering and they brought me back to life. Why wouldn't I be peppy and happy?" She gradually leveled off in her mood after six months, did heavy housework effortlessly but was more interested in quantity production than in quality; flared up more easily at her husband and children than formerly; had a responsible attitude in general toward most practical duties and

church observance. Little concern was shown when her daughter got into trouble although the father went on a rampage. Could sit quietly for hours, or be on the go for long periods. Was irritated when one tried to interrupt her in what she was doing or saying.

Like Brickner's case she took in only part of the total picture. Unlike Brickner's case she was not distractible. She regularly made a bee-line for places, for instance to church, sitting there quietly for an hour before the service began. After church she made a bee-line home without looking to the right or left and without acknowledging greetings of her friends and neighbors. Visited the sick in hospital and friends, as she always had done, but her tireless conversation and interminable visits wore them out. She never gossiped, had no enemies, held no grudges, never got into trouble. Old friends were amused at her simple, amiable, somewhat childish, over-reacting exuberance. Has never appeared anxious, worried, or depressed. It was felt at the time that her defect lay more particularly in her one-trackness, in her inability to be normally distracted.[1] She has remained essentially the same for seventeen years except she is blind now and somewhat more irritable.

The next case is that of a 37-year-old man[2] who at 19 years of age (1932) was brought in to us for treatment and who was discovered to have a massive bilateral frontal lobe defect undoubtedly of congenital origin. Results of air studies were later confirmed by an open flap operation. His unusual behavior dates back to his earliest walking years. He has average intelligence. At the age of 12 (1925) he was diagnosed a "psychopathic personality." Attended high school for three years. He had been studied by Halstead, Gantt, Miles, *et al.* Detailed and careful analysis of his attitude, conduct, and feelings over a period of 18 years indicates that his condition is essentially quite different from that of the classical psychopathic personality, and the concept of "primary social defect"[2] was advanced as the most valid characterization of his condition. The author has never come in contact with a human being who had more reason to become neurotic, psychotic, anti-social or self-destructive, and yet he has never manifested any of these reactions. From earliest days he has met with nothing but frustration, punishment, failure in achieving status at home, at school, in work and in friendship and yet he would like to be successful in all these spheres. The world meets his simple, child-like but impressive smile and manner with a smile which quickly fades and leaves him actually one of the most lonely of creatures, but I doubt if he thinks so. His mood is uniform; his affable manner, vocabulary, and stereotyped expressions are the same today as 18 years ago—all are monotonously unchanged. He never holds grudges nor speaks ill of anyone, never picks a fight, never tricks anyone. He is drawn neither to underworld characters nor to churchmen. One is struck with the childish simplicity and superficiality of his petty lying and stealing and sex experiences which are unpremeditated. Yet these acts apparently are never the result of pure impulse dissociated from their settings. Of all patients encountered he is by far the most stimulus bound. What he does at the moment absorbs his complete attention, and he shows irritation when suddenly asked to shift. His philosophizing is limited to familiar stock phrases such as "a stitch in time," "the early bird catches the worm," etc. He cannot amplify or elaborate the good or the bad. But no one can know him intimately without developing a profound sense of sympathy and a curious feeling of warmth and admiration for his child-like simplicity and guileless responsiveness, in short, his simple cross-sectional friendliness. Viewed from the point of view

of events in time he has, of course, always been a "pain in the neck" to his parents, his school teachers, employers, and neighbors. Everybody knows there is something radically wrong, but no one can put his finger on it. He has no bridges over his streams of activity. With all the painstaking explanation through the years, his parents yet cannot see why he should be so different from everybody else they know, why he cannot hold the jobs he can so easily secure, and why he cannot follow through and deliver. They cannot realize that other people are also baffled and upset by these differences. In short, he is not understood. People just do not know how to make allowances for this unusual type of stranger in their midst. But he goes his busy way, apparently unaffected by it all, reacting to life each day in the same automatic way.

Now let us turn to two cases with prefrontal leukotomy to see what interruption of the subcortical fibres has done to the social and emotional self of these patients. Both have been known to the author for many years before operation.

One, now a 48-year-old woman, has from adolescence met with little success in her struggles for adjustment. Following her debut she worked but became increasingly tense and unhappy. Throughout the years she had had several psychotic breaks, mostly of a paranoid nature mixed with overactivity. Between attacks she supported herself and managed to control her strong ambivalence toward her mother. The breaks became more frequent and violent. She would sit up all night in order to be certain that her mother did not kill her. She was so "tortured" that she threatened suicide.

A frontal leukotomy was performed in 1947. Since that time she has lost all evidences of tortured feelings. She looked at life for the first time in a practical way, decided to live by herself which she could never do before. Now she is friendly to her mother. However, she cannot hold a job although she never worries. Her previous defenses, mostly abnormal, had been impregnable. Now she has little or none and has no insight. She needs to work but lives in bland acceptance of her dependency role. All somatic complaints, of which she had many, have disappeared. She is a restful person now, whereas before she radiated tension. She is uniformly friendly with everyone, always giving presents to people although she cannot afford them, but expects nothing in return. She does not court personal favor, help, or protection.

Here again is shown a simple, uncomplicated social being. But where are even the remnants of the resentments and hostilities piled up over the years through frustration and failure in self-actualization?

Another striking case in this respect and far more dramatic is that of a 32-year-old woman who had been studied and treated by us and others off and on from the age of 8 years. She had had difficulties in adjustment all of her life with several psychotic breaks and hospitalizations. Diagnosis: Psychopathic personality with psychosis, schizophrenic episodes with violent paranoid manic features. She had always been a very immature and highly impulsive girl—during her psychotic periods shamelessly exhibitionistic, dangerous to others, and had once slashed her wrist. Because of her hostile, uninhibited behavior, sexual and otherwise, we naturally approached the subject of leukotomy with reservations. We finally decided to be conservative and do only one side first. After this was done, she immediately became, surprisingly enough, an angel. She was sure a miracle had been performed. She stated: "I couldn't stand

that suffering any longer, I was going to kill myself. . . . Now I am going to be the real person I always wanted to be." But four weeks later she went into the worst psychotic episode she has ever had, which terminated abruptly five months later when the second side was done. The same classical leukotomy technique was used on both sides. Her behavior since then (it is now eight months after the second side was done) can be described as shy, quiet, colorless, placid, almost apathetic, but friendly and mildly affectionate, stereotyped, and very correct. Everybody is drawn to this fragile, passive child. Previously, especially in her more active periods, she had been hypercritical, vitriolic, colorful, and extremely shocking in her crude but clever humor. People laughed, but withdrew. Now she is a household pet and a faded angel indeed compared to the four-week period immediately following the unilateral operation. I do not think that the first operation did anything for her. I have seen her in quiet, contemplative moods prior to attacks of excitement. Once, at least, she had a retarded depression for several months. It is of interest that previous to the operations her room was as chaotic and colorful as her behavior, with pin-up girls and men, flaming drapes, scattered books, and effects. Today her room in the convalescent home is bare and uninviting, not at all from necessity. Except for her few little possessions arranged neatly on the dresser, it is the same as the day she entered it. Before the operation she had violent crushes and quarrels with nurses and female patients and was wantonly promiscuous with men. Since the operations she states that sex has no appeal for her. Previously she often spoke of herself as being two people in one—"a devil and a saint, Dr. Jekyll and Mr. Hyde." Now she says, "I am just myself." Knowing her from childhood up it is not difficult to see what she means here. Her grasp of reality was always so weak that she could easily by-pass it and one moment identify with all that was good in people, punishing herself for acting out, only to act out again. The operations have very likely cut down the over-elaborations of both selves, leaving her childhood aspirations quiet and undisturbed. It will be most interesting indeed to know how she fares in the years to come.

Freeman[14] ably discussed the role of the frontal lobes in the development of what he terms "social skills." He believes that higher social skills are definitely affected in frontal leukotomy, certainly those skills that are particularly important and noticeable, for instance, in the practice of professions that deal especially with people. Many of his patients following leukotomy have carried on skilled trades and work requiring high technical ability, but he has not seen preservations of these highest social skills.

It must be remembered that we are not interested for the purpose of this paper in the merits of any operation which is done for the relief of mental suffering in man already handicapped by disease. We are interested in the meaning of it all in terms of brain functioning. Up until recent years medical men and surgeons have hesitated to operate on the frontal lobes of man for fear it would release man's strong animal drives, such as sex and aggression, making him a menace to society, medical theory to the contrary. Hughlings Jackson long ago laid down the scientific theory of evolution

and dissolution that only now is being given adequate clinical test by man. Man's high social performances are undoubtedly forged in the workshop of the great nerve nets of the brain with special accent upon the prefrontal areas. There are represented experiences of the good and the bad, the loves and the hates, the false and the true. Out of his reaction to and preoccupation with frustration, conflict, and struggle made possible by an intact brain, is fashioned man's bed for weal or woe.

It may be that the scientific spirit within us still prefers to cling securely to the more concrete minutiae, letting others deal as they may with abstract principles as they affect the whole man in his universe. However, I am of the opinion that a clinician at some stage of his work should not only consider the latter his privilege, but also his obligation. The following statement, therefore, is pointed in this direction. That mankind is actually as well as potentially more good than bad, more constructive than destructive, more mutually aiding than mutually competing—in short, more social than antisocial—is in no way disproved by any of these man-made or nature-made experiments. By the same token it can be our reasonable hope that war is the result of overfeeding man's mind on prejudices, superstitions, and misconceptions rather than the impact of the simple impulses of his heart.

The evidence to date seems to point to the frontal lobes as the seat of distance awareness, anxious concern, and motivation. Drives leading to adequate motivation are essential to man in coping effectively with the world in which he finds himself. If the great nerve net enables man to develop, amplify, and elaborate the processes of thought and feeling in many conflicting directions, it may be that here lies the source of sustained tension. It may be that such tension is necessary to activate the energy producing systems of the organism to create these drives without which man cannot achieve self-realization as a successfully contributing social being.

There has been no attempt in this discussion to disagree with existing scientific theories concerning frontal lobe functioning. Intelligence as a basic function of the brain is represented throughout the entire cerebral cortex. That it is not represented equally throughout seems at least obvious from the evidence in hand today, both laboratory and clinical, with the exception of the work of Lashley and his followers on animal material. However, Halstead's[9] statement that "intelligence (biological intelligence) is dis-

tributed in a gradient with its maximal representation occurring in the cortex of the frontal lobes" coincides with this. But he also holds that no evidence for a general principle of mass action is yielded by his study.

We must, however, not forget the great strengths and weaknesses that man is born with which play an important part in his survival. Current studies on man's predispositions to favor the focal rather than the peripheral (Gesell[6]), the perceptual approach to life over the conceptual, and vice versa now being carried on by Carney Landis and his associates[8, 14] at Columbia are very provocative indeed. They not only give clues as to which patients may more readily find their way back from the unrealities of mental illness regardless of the specific treatment employed, but also give hints for the early recognition of such predispositions for purposes of prevention and the development of sounder mental health.

In general then, let us postulate that man's greatest pain comes from coping with the confused shadowy conceptual peripheries, and man's deepest pleasure can come on the return trip to the perceptual center with nuggets of wisdom in his hands. For what is periphery today can be "central" tomorrow. What is "central" is the more surely known, giving man a degree of security and comfort as nothing else can do. It may well be that by blocking the traffic lanes to the periphery the surgeon relieves not only mental, but physical pain or, perhaps more properly stated, the spreading dread of pain in general leaving the patient freer to deal with the simpler, unamplified, more benign, and less intense everydayness of things.

Normal man, too, must accept the challenge to move freely between his central straight-aheadness and his more remote periphery if the technician in him is ever to become the poet and the philosopher achieving together greater and greater social awareness, creativity, and responsibility. This man can do if the great association areas of his brain are intact. This man cannot do if free access from the center to the periphery and back again is denied him.

I wish to acknowledge here my deep indebtedness to all those who have so generously shared with me their thinking as well as their findings in the preparation of this paper.

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COMMENTARY ON TYPHUS CONTROL IN WORLD WAR II

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This article for the volume to be published on the occasion of the retirement of Dr. Winternitz as Professor of Pathology is one of the final entries of a journal that was opened under his instruction about thirty-five years ago. Although the writer, as a medical student and resident, had received unforgettably vivid teaching from Dr. Winternitz during the period from 1911 to 1915, the associations with which this article deals began formally in 1916. In that year, after a few months of training under Dr. Hans Zinsser, I was put in charge of the new Laboratory for Bacteriology on the Fifth Floor which Dr. Winternitz had recently added to the old Pathology Building at the Johns Hopkins Medical School. In the original "protocol" of the experimental program, and of the even more experimental appointment, there was no mention of typhus fever and little, if anything, about medical administration. There were, of course, no indications of the unforseen events of the future. Nevertheless, the apparently accidental arrangements of those days started the series of happenings that finally included the opportunity to take part in the administration of typhus control in World War II. It is now clear that throughout the haphazard occurrences of the years since 1916 a main element in the Fifth Floor protocol, although unwritten, was the influence of Dr. Winternitz's philosophy and conceptions of relationships among people, communities, and ideas. Typhus and typhus control involve these relationships through the natural history of the disease and the social and economic implications of its prevention. Hence, a pupil of Dr. Winternitz working in that field would be especially aware of his influence, as well as of his example and contributions. It is with recollections going back to Baltimore before our country's entry into World War I, with thoughts of unbroken association over the years, and with awareness of his influence that these observations on typhus control in World War II are offered for this volume dedicated to Dr. Winternitz.

The word "commentary" in the title of this paper is intended to mean an attempt to generalize from specific instances and to venture a few opinions

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and speculative remarks relative to the control of louse-borne, exanthematic typhus fever that was achieved during World War II. Details and documentation might well be demanded by the reader. Fortunately, the scientific work, outlines of the administrative arrangements, and descriptions of the main episodes have been published. The few selected references[1-11] at the end of this article must serve as a guide to the larger literature. The whole undertaking, however, was such a vast collaboration of individuals and agencies in democratic nations engaged in a total war effort that neither documentation nor adequate acknowledgement is possible in a paper of this sort. An article published in 1948[3] contained names and mention of many individuals and military and civilian agencies that were devoted to this collaboration. It is admittedly incomplete. A full account is planned for publication in a volume connected with the medical history of the war. Meanwhile, the writer begs the informed reader to fill in the details and also to understand that while his immediate opportunity to participate in this work came from connection with the Army, he is fully aware of all associations with other Services and agencies.

Two main conclusions have been drawn from the results of the attack against typhus during the war. One is that American troops were effectively protected against typhus. The other is that epidemics of typhus fever among civilian populations were brought under control and stopped. It is of some interest to examine both claims.

During the war, from 1941 to 1945, there were only 64 cases of epidemic, louse-borne typhus fever among American troops and no deaths. The chief specific preventive measures employed among troops were those directed against louse infestation and procedures for the establishment and maintenance of immunity by injections of typhus vaccine. Contributory general preventive measures were those of personal hygiene, cleanliness, good nutrition, proper housing when available, avoidance of contact with known sources of infection, and education. The average age group of the troops was a favorable element against fatality, but their previous lack of exposure to typhus had given them no opportunity to acquire immunity naturally. Where so many general factors are contributing to a final result it is difficult to assess the value of specific measures. However, it appears possible to do so with regard to anti-louse measures and vaccination against typhus.

With regard to the value of anti-louse measures in protection against typhus no question of principle was raised or has been raised. Since 1909, when Nicolle proved that the body louse transmitted epidemic typhus among human beings, it has been obvious that prevention of infestation with lice

and delousing of the infested would prevent the spread of typhus. The rare exceptions were infections acquired by inhalation of infected louse feces or by the introduction of infected louse feces into the conjunctival sac. The German and Austrian armies in Central Europe in 1915 demonstrated the fact that by bathing and steam disinfestation of clothing the occurrence of typhus among troops could be held to a low level in the midst of even so large a civilian outbreak of the disease as the Serbian epidemic. However, later, during World War I, troops on the Western Front with a high rate of louse infestation and suffering from outbreaks of the louse-borne rickettsial disease, trench fever, were free from typhus. No doubt this apparent mystery is to be explained by low incidence of exposure to typhus.

Although there was no question of principle involved, there was a crucial decision to be made relative to delousing methods and materials about 1943 when evidence of the effectiveness of new insecticide powders was coming in from reports of extensive investigations of the Department of Agriculture, and the International Health Division of the Rockefeller Foundation and the Army. The first of these preparations was the pyrethrin containing MYL, and this was soon followed by DDT (used as a powder of 10 per cent dichlorodiphenyltrichlorethane in pyrophylite). Both were effective; DDT superior. Contradictory reports of field tests made by the U.S.A. Typhus Commission at Cairo were corrected by further trials. Largely through the clear sight of Colonel William S. Stone a correct evaluation of the evidence was reached and the decision was made to rely on anti-louse powders, with persistent action, in place of cumbersome steam disinfestors. Later in 1943 the demonstrations of mass delousing by dusting civilians with DDT powder, carried out by Dr. Fred L. Soper and the Rockefeller Foundation Team in North Africa, placed this method on a firm basis. Thus the prevention of louse-infestation by the use of insecticidal powder became not only a safeguard of troops against typhus but also a revolutionary advance in the control of epidemics of typhus among civilians.

For the appraisal of the value of a vaccine there is no formula as simple as the one used in the section on delousing where it could be said that freedom from lice meant practical freedom from typhus. Since many variables and intangible factors were involved, it is more difficult to estimate the actual efficacy of vaccination against typhus.

By the end of 1941 there was ample evidence from the work of Weigl, Zinsser, Cox, and others that animals and human beings could be made resistant to typhus infection by injections of suspensions of killed *Rickettsia prowazeki*. This was sufficient to warrant the adoption of typhus vaccina-

tion by the Army late in 1941. The first preparation used in 1942 was the Cox yolk sac vaccine. It was known that the original Cox vaccine was not as good an antigen as desirable. Hence individual and collaborative efforts were stimulated to produce a better vaccine. Great improvements were made in 1942 by the ether extraction method discovered by Craigie and by the addition to the vaccine in 1942 of the soluble specific substance discovered by Topping and Plotz. Results obtained with the earlier vaccine need not concern us here, although it is recalled that soldiers who received the original preparations were believed to have acquired some immunity. Tests with the improved vaccine, carried out by the U.S.A. Typhus Commission among Egyptians at Cairo in 1943 and 1944, showed definitely that while the vaccine did not protect against infection, it did produce a state of resistance that greatly ameliorated the disease. The vaccine came to be regarded chiefly as a mitigator and in a sense as insurance against fatality. The evidence indicates that the mild, often almost unrecognizable, cases among vaccinated American troops, and the absence of deaths were attributable to resistance acquired from vaccination.

Although it was shown that lice could be infected by feeding upon a vaccinated person suffering with typhus, there was no direct evidence that the spread of the disease was aided by the obscure, often unrecognizable, cases of typhus occurring among the vaccinated. It appears to be a reasonable assumption, on the other hand, that vaccination against typhus prevented an undeterminable number of infections. This inference, unprovable for lack of data, is drawn from the later knowledge that the exposure of American soldiers to louse-infested typhus victims exceeded all anticipation. This was true in Egypt, Iran, North Africa, Italy, Japan, and Korea, and possibly in Germany. In some of these areas there were estimated to be four or more times as many cases as those reported, indicating incidence in terms of tens of thousands of cases and consequently high risk of exposure. The degree of exposure of troops in the North African campaign was not well known until months after the landings of 1942. The fact that they had been vaccinated against typhus gave some measure of reassurance. Of course, how often the vaccine protected against infection, no one will ever know. An indication of what might have happened if vaccination had not been employed is given by the experiences of an unvaccinated British unit at a port at which vaccinated American soldiers were engaged in similar duties. Among these British troops there were 36 cases of typhus with a case fatality rate of 32 per cent. During the same period there were 9 mild cases among all American troops in North Africa and no deaths.

Reviewing the total experience at this date after the war, the field experience confirmed the opinion expressed at the National Research Council Conference on Typhus in June, 1944. This opinion is that while vaccination against epidemic louse-borne typhus does not always prevent infection, it causes the disease to be mild and appears to prevent death from typhus.

The use to be made of typhus vaccine in the control of an epidemic was a debatable question at one time. As it takes several weeks for the production of a serviceable state of immunity after the injection of the vaccine, it is obvious that immediate effects upon an outbreak would not be expected. A vaccination program carried out at the start, however, might be expected to make the decline of an epidemic more abrupt and certainly might be expected to save lives. In the author's opinion, the fear of aiding the spread of the disease by creation of many mild cases of typhus among the vaccinated is unwarranted, and without any proof from field experience. During the war it was agreed that vaccination should not be attempted on a mass scale among a population afflicted or immediately threatened with an epidemic, but had best be used for the immunization of all "key personnel," officials, clergy, bankers, transportation workers, and others rendering essential services, as well, of course, as all medical and sanitary personnel engaged in the work of control of the epidemic and care of the sick. Finally, it is not to be forgotten that one of the greatest values of vaccination against typhus is the protection of the workers in laboratories and plants where typhus vaccine is tested and manufactured.

During the war and the first eight months following the surrender of Japan, from December 1943 to May 1946, the new preventive and control measures were applied with the backing of military authority against three epidemics of typhus fever among war-wracked civilian populations. The first test was in bombed, chaotic, overcrowded Naples from December 1943 to February 1944.[9, 11] The second was in the Inner Reich of Germany, in areas in which the typhus-ridden inmates from Nazi prison camps had scattered from March to June 1945.[4] The third was in disorganized Japan, flooded with repatriates coming in from Korea, in the winter of 1945 and early spring of 1946.[8] Conditions varied in each epidemic, but the principles of control were essentially the same as those laid down at the League of Nations Typhus Control Conference in 1937, namely, case finding, isolation of cases and regions (*cordon sanitaire*), delousing, and immunization. The methods and materials were the new products of work done during the war. These were chiefly organization and operation of units of trained personnel, mass delousing by dusting with DDT powder fully clothed civilians,

some use of typhus vaccine, and the maintenance of the reporting systems that constitute medical intelligence. In each of these epidemics, the peak of cases was reached at a date earlier than would have been expected to occur in the absence of control measures, the number of cases was far less than reasonably might have been anticipated, the decline of the outbreaks was relatively abrupt, and the secondary foci or small coincident local outbreaks were promptly extinguished. The epidemiological data on which these statements are based are to be found in the publications cited. The conclusion is warranted that for the first time in the history of typhus in wartime, epidemics of the disease were brought under control and stopped before they had run their previously customary course.

The control of typhus fever achieved during World War II deserves to rank among the foremost achievements of military preventive medicine. The achievement freed military operations from the hampering effect which typhus in the past had exerted upon campaigns—an effect which had occasionally determined the outcome of a war. The significance of the achievement for civilian preventive medicine and public health is even greater. While the time may be long before typhus is eradicted from its ancient endemic regions, the statement may now be made with assurance that given the means and the will to use them no country need ever again suffer from a devastating epidemic of typhus fever.

There were peculiarities in the behavior of typhus fever between the wars, and these peculiarities should be noted as background against which to judge the claim that control of the disease was gained in World War II. Following the severe epidemics that occurred in Eastern Europe during World War I, there were epidemics with cases estimated in the millions in Roumania, Poland, and Russia in the years 1918 to about 1922. During the decade from 1923 to 1933 there was a steady decrease in the prevalence of typhus in Europe and Russia. In Italy there had been no case of typhus reported in the 15 years from 1928 to 1942, and only 54 cases in the six years prior to 1928. A generation of non-immunes had grown up in that country. One may suppose that constant anti-typhus campaigns, wholesale destruction of lice, improvement of the economic conditions of people, and a cessation of national wars were the causes of this decline of typhus. However, it must be admitted that the still mysterious factors of epidemic periodicity and "epidemic constitution" may have been operating to produce the result. During the next seven years, 1933 to 1940, however, a new wave of typhus occurred in Egypt, North Africa, South Africa, Roumania, Poland, Yugoslavia, and in parts of the Soviet Union. There were outbreaks also

in Chile and Portugal. By 1941 Spain, usually free from the disease, was involved. When the European war broke out in 1939, typhus was on the move again, and when the United States entered World War II in 1941, there was ample reason for the fear that this most tremendous and destructive of all wars might be accompanied by epidemics of typhus fever as severe, if not more devastating, than any in the long history of this major cause of human misery. This fear was increased by uncertainties due to the breakdown of international epidemiological reporting after 1939, and the consequent low state of medical intelligence (epidemiological information). As we look at the total situation in retrospect we feel justified in saying that these things might have happened, but did not happen because great scientific advances in the knowledge of typhus and possible means of control had been made between the wars, and because vigorous co-operative actions were taken to speed up improvements and to apply effective preventive measures with the full force of military authority supported by democratic peoples united in a common purpose.

Many individuals, agencies, and organizations joined wholeheartedly in the wartime attack against typhus. For many reasons, one of the organizations of particular interest was the United States of America Typhus Commission, established by Executive Order No. 9285 by President Franklin Delano Roosevelt on December 24, 1942. The account of the origin of this Commission from a long series of conferences held by Brigadier General James S. Simmons, Chief of the Preventive Medicine Service, Office of The Surgeon General, U. S. Army, starting early in July 1942, when the North Africa landing was in the planning stages, has been published.[2] It is recalled that the leaders associated with him were Dr. Rolla E. Dyer, Chief of the National Institute of Health of the United States Public Health Service, Rear Admiral Charles S. Stephenson, (MC), U. S. Navy, Chief of the Preventive Medicine Division of the Bureau of Medicine and Surgery of the Navy, and later Brigadier General Leon A. Fox. A host of names deserves to be mentioned, but space does not permit the inclusion of the distinguished roster. These names, however, indicate the main characteristic of the Commission, which was a union of the Services for a common purpose.

The establishment of commissions to deal with special problems of national concern has been a time-honored procedure of the Government of the United States and other governments. In civilian experience, and in the experience of the Public Health Service, the Navy, and the Army, there are numerous examples of commissions established to investigate diseases

and to recommend and apply measures for their control. To cite cases from the experience of the Medical Department of the Army alone, particular mention may be made of the Yellow Fever Commission of 1901, the Pneumonia Board of 1918, and the Army Epidemiological Board of World War II. The Army Epidemiological Board, established in January 1941, as the result of plans and recommendations of General Simmons, "for the investigation and control of influenza and other epidemic diseases in the Army," had successfully combined expert civilian and military activities, and had been operating both as a War Department agency and as a division of Preventive Medicine Service of the Office of The Surgeon General. The establishment of the United States of America Typhus Commission was, therefore, in accordance with tradition and current practice. However, the U.S.A. Typhus Commission had some additional characteristics that were derived from the unusual potential dangers of typhus and from the economic and political history of this disease.

Executive Order No. 9285[2] was broadly conceived. It expressed the Government's realization of the importance of typhus fever as a threat to the safety of soldiers, a possible hindrance to military operations, and a probable cause of devastation among the civilian populations with which the armed forces were in contact and for which they would have dependency responsibilities under military government in liberated and occupied countries. In accordance with the estimate of the risks, the Executive Order gave the Commission commensurate scope, responsibilities, and authorizations, under the Secretary of War. It invoked and directed the assistance and support of the Commission by numerous military, executive, and governmental agencies to effectuate a program for the study and control of typhus fever both within and without the United States when typhus fever threatened or might threaten the military population. While making no change in the special activities of the Services, the Executive Order brought together the Army, Navy, and Public Health Service in a joint attack upon typhus. In language characteristic of a democracy even in war, the Order directed the Commission to "secure cooperation" and to "collaborate" with all appropriate organizations.

This is not the place to summarize the staff studies that preceded the issuance of the Executive Order, or to outline the system of approvals and channels that had to be developed to handle the affairs of the Commission, or discuss the many personal and operational problems that were solved in one way or another. Suffice it to say that these were exhaustive, intricate,

and novel to the extent that the Commission was anomalous among military-medical agencies for which no previous provisions had been made.

The field of interest was interpreted to include epidemic (louse-borne) typhus, endemic (murine, flea-borne) typhus, and scrub typhus (mite-borne) tsutsugamushi disease. Through this mandate, the Commission became engaged in the unforseen crises of a global war in all areas in which rickettsial diseases were a threat to military operations. The Commission, with headquarters in Washington, worked not only in the United States, but also in all the chief theaters of operations overseas. Some of the activities of the Commission will be briefly summarized because of their general interest and implications.

In a small way the Commission was an early example of unification of the Services. Its personnel, commissioned and non-commissioned, was detailed from the Army, Navy, and Public Health Service. It was a composite unit under the War Department. There was no great difficulty in the management of the affairs of officers and enlisted men, in accordance with the basic requirements of their respective Services while they were serving with the Commission.

Through the terms of Executive Order No. 9285 and the support and approval accorded by higher authority, the Director of the Commission, a medical officer, had direct access to the chiefs of government departments, including the Secretary of War and the Secretary of State. This privilege of direct communication included also relations between the Director and the General Staff, the special staff of Civil Affairs, the Army Service Forces Staff, and the offices of Commanders of Theaters of Operation. Direct communication on behalf of the Commission was made possible with all agencies concerned in this country, with several foreign missions, and with many individuals. The advantages for effective and expeditious operation were enormous.

Although the Commission had no budget of special appropriations, it was in a position to request and support requests for large expenditures for materials needed for typhus control. It assisted in obtaining priorities and funds for the rapid manufacture of DDT. It was instrumental in placing "firm requisitions" for millions of doses of typhus vaccine at a time when there was a risk that manufacture of the vaccine might have to be curtailed, with consequent dispersal of the specially trained and immunized personnel working on vaccine production at commercial plants. The Commission was able to expedite, at critical moments, the transportation by sea and air of

supplies to combat typhus, and at all times to assist in the preparation and assemblage of anti-typhus supplies.

During the war the assembling and holding of trained expert personnel for particular purposes was a constant problem because of the general manpower needs of the country. Through the Commission it was possible to maintain a unit of experts on typhus and to preserve civilian laboratory organizations engaged in work on typhus.

Through privileges of representation of higher authority the Commission was able to assist in matters of general diplomatic and governmental nature. It had mutually beneficial relations with Civil Affairs and with military commissions and governments in foreign countries, with the Lend Lease Administration, with the United Nations Relief and Rehabilitations Administration (UNRRA), and with similar agencies. At the Cairo Conference in 1943 the Commission, represented by General Fox, was instrumental in securing free distribution of typhus vaccine to Middle East countries. Through General Fox, a group of officers of the Commission was admitted to Yugoslavia in 1944, and an officer of the Commission accompanied the British Military Mission into Greece.

The Collected Reprints 1943-1947[10] of the Commission present most of the original scientific work done on typhus and related rickettsial diseases by members of the Commission. Additional publications have been made since 1947. Well-equipped laboratories staffed by experts were established and maintained by the Commission in Cairo, Egypt, and in Assam-Burma. In addition to typhus materials returned to laboratories, specimens of rare plants and animals were sent from these stations to museums in this country. Furthermore, the Commission had the privilege of assisting in obtaining facilities and support for laboratory investigations of typhus in the United States and England.

In the episodes of the control of the typhus epidemics among the civilian populations in Naples, the Inner Reich, and in Japan, summarized above, are to be found the best examples of the value of such an organization as the Commission. Supported by military authority, and with the collaboration of other agencies, it served effectively in curbing these dangerous outbreaks of the disease.

While the material for this review has been drawn almost entirely from the experience of the United States of America Typhus Commission, the commentary has not been intended to be so centered. The details are to be regarded as illustrations of some of the general aspects and meaning of the control of typhus.

In the past typhus has determined the outcome of military campaigns and has affected the economy, morals, and social institutions of communities. As a result of control over the disease gained during World War II these effects of the disease need not occur again. The spectacular role of effective preventive medicine in a crisis is apt to be forgotten when the continuation of safeguards becomes the routine observance of familiar precautions. But the influence of preventive medicine upon the course of events persists and will remain a power, however inconspicuous, in keeping typhus within bounds. The restraint of typhus may be as effectively accomplished by national effort and international collaboration in peace as it was during the war. The results afford another example of the world-binding influence of international epidemiology and control of infectious disease. It is trusted that forces of peaceful international associations were strengthened by demonstrations of the control of typhus fever in World War II.

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CLINICAL OBSERVATIONS ON TERRAMYCIN*†

FRANCIS G. BLAKE, GEORGE J. FRIOU, AND ROBERT R. WAGNER

In a recent report, Finlay, Hobby, and their collaborators[1] have described a new crystalline antibiotic, terramycin, isolated from broth cultures of a new actinomycete, *Streptomyces rimosus*. *In vitro* studies[2] of terramycin and its salts have shown that this antibiotic in low concentrations is a highly effective bacteriostatic agent against a wide variety of gram-positive and gram-negative bacteria. *In vivo* observations[3] have also shown that it is a highly effective therapeutic agent against experimental animal infections with terramycin-sensitive organisms. It possesses low toxicity.[1,3] Studies[4] on absorption and excretion in dogs and rabbits have shown that terramycin salts are absorbed readily following either oral or parenteral administration, the most satisfactory results having been obtained on oral administration of the hydrochloride. It would appear from the foregoing experimental data that terramycin should be an effective antibiotic agent for the treatment of a variety of infections in man.[3,4] A therapeutic evaluation has, therefore, been undertaken,[5] in which we have had the opportunity to participate.

The present report records our clinical experience to date with the use of terramycin in fifteen patients with various infections.

Methods

Selection of patients. The patients selected for treatment were arbitrarily chosen to represent a considerable variety of infections, in some of whom other chemotherapeutic agents had been ineffective.

Dosage. Terramycin hydrochloride administered orally was used in all cases. An initial or loading dose of 1.0 gm. q.h. for three doses was given in 10 patients, of 1.0 gm. in 5 patients. Subsequent treatment varied from 3.0 to 6.0 gm. per day given in 0.5 gm. or 0.75 gm. doses q.3 h. or q.4 h. depending on the nature of the illness and response to treatment. In seven instances the dosage was reduced to 0.25 gm. q.4 h. toward the end of

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† The terramycin used was supplied by Chas. Pfizer & Co., Inc., Brooklyn, New York, through the courtesy of Dr. Gladys L. Hobby.

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TABLE 1

CASES TREATED WITH TERRAMYCIN

Case	*Diagnosis*	*Bacterial etiology*	*Sensitivity μg./cc.*	*Terramycin dosage (gms.)*				*Side effects*			*Result*
				Initial	*Subsequent*						
					gms.	*q.-h.*	*days*	*n.*	*v.*	*d.*	
J.S.	Acute bacterial endocarditis	*Staph. aureus*	2.0	1.0 q.h.3x	0.5 0.5 0.75	4 3 3	14 3 36	±	—	—	Cured
W.P.	Subacute bacterial endocarditis	*Strep. fecalis*	2.0	1.0 q.h.3x	0.5 0.75 0.5	3 3 3	9 19 17	++	+	±	Temporarily improved. Relapsed following discontinuance of terramycin
E.F.	Subacute bacterial endocarditis	*Strep. viridans*	1.0	1.0 q.h.3x	0.5 0.75 0.5 0.75 0.5	3 3 3 3 3	2 12 6 5 12	±	—	±	Temporarily improved. Relapsed following discontinuance of terramycin
C.P.	Lobar pneumonia	D. *pneumoniae*	1.0	1.0 q.h.3x	0.5	4	6	—	—	—	Cured
A.B.	Lobar pneumonia	D. *pneumoniae*	0.5	1.0	0.5 0.25	4 4	4 2	—	—	±	Cured
G.P.	Lobar pneumonia	D. *pneumoniae*	0.5	1.0 q.h.3x	0.5	4	5½	—	—	±	Cured
A.T.	Lobar pneumonia	D. *pneumoniae*	0.5	1.0 q.h.3x	0.5 0.5	3 4	3½ 4½	±	±	+	Recovered

Case	*Diagnosis*	*Bacterial etiology*	*Sensitivity µg./cc.*	*Terramycin dosage (gms.)*				*Side effects*			*Result*
				Initial	*Subsequent*						
					gms.	*q.-h.*	*days*	*n.*	*v.*	*d.*	
E.S.	Urinary tract infection Bacteremia	*E. coli* *B. prot. vulgaris* *Strep. viridans*	4.0 1.0	1.0	0.5 0.5 0.5 0.25	2 3 4 4	(12x) 3 1½ 19	±	—	—	Bacteremia and bacilluria cleared but reinfected with *E. coli*
C.S.	Urinary tract infection	*E. coli* *B. proteus*	4.0	1.0	0.5 0.25	4 4	4½ 5	—	—	++	Bacilluria cured
E.D.	Urinary tract infection	*B. muc. capsulatus*	5.0	1.0	0.5 0.25	4 4	4 2	±	±	±	Bacilluria cured
J.G.	Urinary tract infection	*B. muc. capsulatus*	5.0	1.0	0.5	4	10	++	++	—	Bacilluria diminished
A.P.	Pyoderma	*Staph. aureus* *Staph. albus*	1.0 0.5	1.0 q.h.3x	0.5 0.25	3 4	7 1	±	±	±	Cured
J.A.	Fusospirochetosis	Fusiform bacilli Spirochetes		1.0 q.h.3x	0.5 0.25	3 4	4 1	—	—	±	Cured
R.S.	Influenza			1.0 q.h.3x	0.5	3	2½	—	—	—	No effect
G.G.	Cholangeitis lenta	*Strep. viridans*	2.0	1.0 q.h.3x	0.75 0.5	3 4	8½ 4½	—	—	—	Cured

treatment. In one patient (Case E.S.) the initial dose of 1.0 gm. was followed by 0.5 gm. q.2 h. for 12 doses.

Sensitivity. The sensitivity of the infecting microorganisms to terramycin hydrochloride was determined in 13 cases by the method described by Hobby *et al.*,[8] the dilutions used providing end points of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 50.0 micrograms (μg.) per cc. Since it is obvious that these end points are fairly widely spaced, the sensitivity may have been somewhat greater than that recorded. The results are recorded in the case reports.

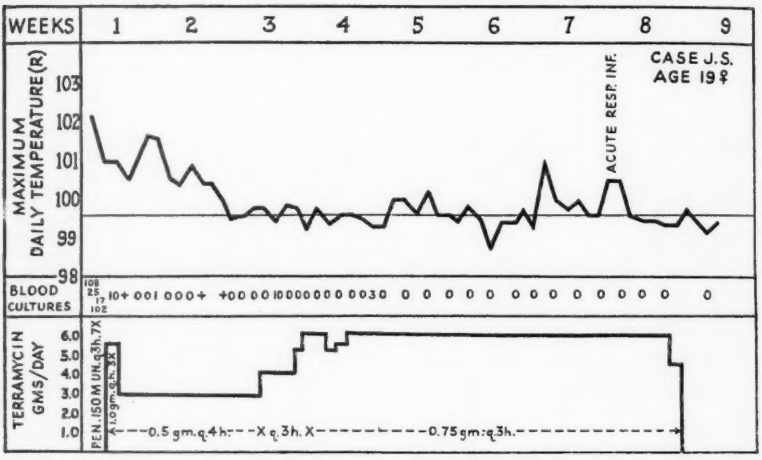

CHART. 1. Case J.S. *Staphylococcus aureus* septicemia and mitral endocarditis.

Case reports

The patients selected for treatment with terramycin were 1 case of acute staphylococcal endocarditis, 2 of subacute streptococcal endocarditis, 4 of pneumococcal lobar pneumonia, 4 urinary tract infections, and a miscellaneous group of 1 case each of staphylococcal pyoderma, buccal and penile fusospirochetosis, influenza, and cholangeitis lenta. Pertinent data concerning them are presented below and summarized in Table 1. Selected cases are further illustrated in Charts 1-6.

Case J.S., age 19, female. Chart 1. *Staphylococcus aureus* septicemia with acute mitral endocarditis and embolic left-sided hemiplegia. This patient, the growth of whose *Staphylococcus aureus* was inhibited by 2.0 μg./cc. of terramycin, by 4 un./cc. of penicillin, but not by 5.0 μg./cc. of chloromycetin, was admitted on February 8,

1950, severely ill and semicomatose, on the ninth day of disease, after having failed to respond to intermittent treatment with sulfadiazine and penicillin at home. Having further failed to respond to 1,050,000 units of penicillin, terramycin therapy was instituted on the 11th day of illness and continued for 53 days. Progressive gradual improvement ensued. The temperature became normal on the 12th day of treatment, no further embolic phenomena occurred, and the hemiplegia gradually disappeared except for residual weakness of the left foot. Although the staphylococcemia promptly abated under terramycin therapy, it should be noted that blood cultures were occasionally positive for *Staphylococcus aureus* during the first four weeks of treatment and became permanently negative only after the dose of terramycin had been increased to 6.0 gm. per day for a week. Whether the increase in dosage or the eventual healing of the

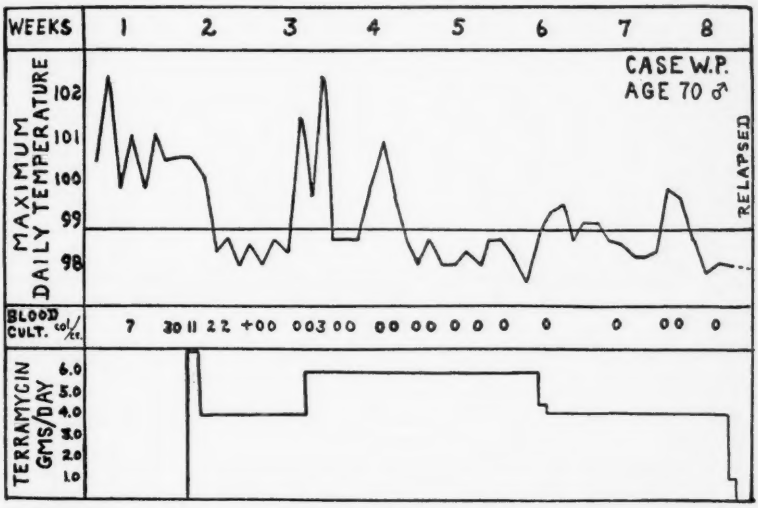

CHART 2. Case W.P. Subacute bacterial endocarditis due to *Streptococcus fecalis.*

mitral endocarditis was responsible for this is uncertain. At the time of discharge on the 59th hospital day, recovery was apparently complete except for a persistent apical systolic murmur and weakness of the left foot.

Case W.P., age 70, male. Chart 2. Subacute bacterial endocarditis due to *Streptococcus fecalis*, the growth of which was inhibited by 2.0 μg./cc. of terramycin but not by 10 μg./cc. of chloromycetin, 100 μg./cc. of streptomycin nor 5 un./cc. of penicillin. This patient, admitted on February 2, 1950, approximately one month after the onset of his illness, was started on terramycin therapy on March 15, after the diagnosis had been established by the appearance of an apical systolic murmur, a palpable spleen, scattered sparse petechiae, and repeatedly positive blood cultures. As shown in Chart 2, the original dosage of 4.0 gm. per day was probably inadequate. On the 11th day of

treatment the dose was increased to 6.0 gm. per day, being maintained at this level for 19 days. As a result, the temperature returned to normal and repeated blood cultures remained sterile. On the 30th day of treatment, because of anorexia, nausea, and occasional vomiting, the dose was reduced to 4.0 gm. per day and was maintained at that level until the 47th day, when treatment was discontinued. Beginning six days later, however, blood cultures again became positive for *Streptococcus fecalis*, and it was apparent that the infection had not been cured.

Case E.F., age 62, male. Chart 3. Subacute bacterial endocarditis due to *Streptococcus viridans*, the growth of which was inhibited by 1.0 μg./cc. of terramycin, by

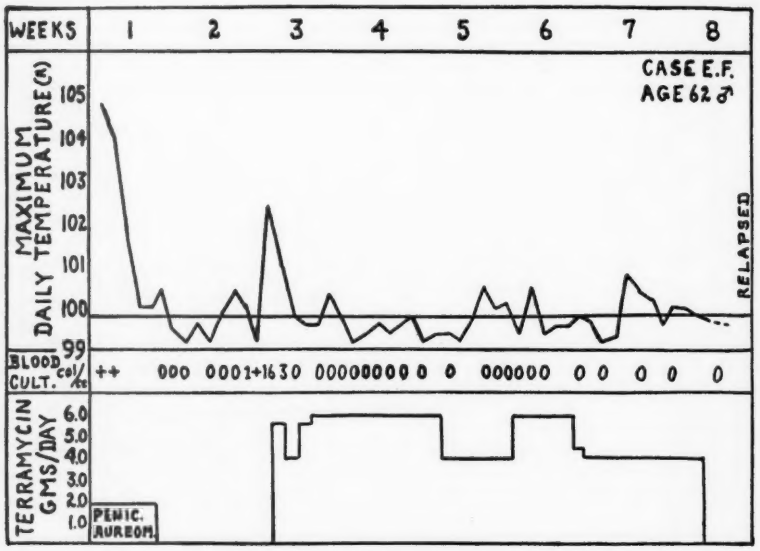

CHART 3. Case E.F. Subacute bacterial endocarditis due to *Streptococcus viridans*.

0.03 un./cc. of penicillin, but not by 5.0 μg./cc. of chloromycetin. This patient with chronic rheumatic heart disease and mitral stenosis was admitted on March 8, 1950, approximately 2½ months after the probable onset of his disease and following an embolic splenic infarction. Because of his serious condition he was immediately treated with penicillin and aureomycin before the diagnosis was established or the result of the initial blood cultures was known. Following the initial prompt clinical response to treatment he was permitted to go without further medication until the suspected diagnosis of subacute bacterial endocarditis could be fully confirmed by the reappearance of bacteremia and fever. As shown in Chart 3, this occurred at the end of the second hospital week. Terramycin therapy was then instituted and continued for 37 days. Blood cultures became sterile, and there was progressive clinical improvement.

The occasional moderate elevations of temperature during the course of treatment are quite similar to those observed during the successful treatment of subacute bacterial endocarditis with penicillin[2] and were not considered to be necessarily indicative of inadequate dosage or an unsatisfactory therapeutic response. On the 13th day of treatment he developed blurring of vision in the right eye, which appeared to be due to embolism of a retinal arteriole, and in the fifth week mild symptoms suggesting a splenic infarction occurred. Comparable events may occur during penicillin treatment without serious consequence or evidence that the emboli are infected.[2] Eight days after terramycin was discontinued, however, blood cultures became positive again for *Streptococcus viridans*, and clinical relapse occurred.

Case C.P., age 36, male. Chart 4. Pneumococcal lobar pneumonia, right lower lobe. Admitted on the 2nd day of disease, acutely ill, blood culture negative. Pneumococcus

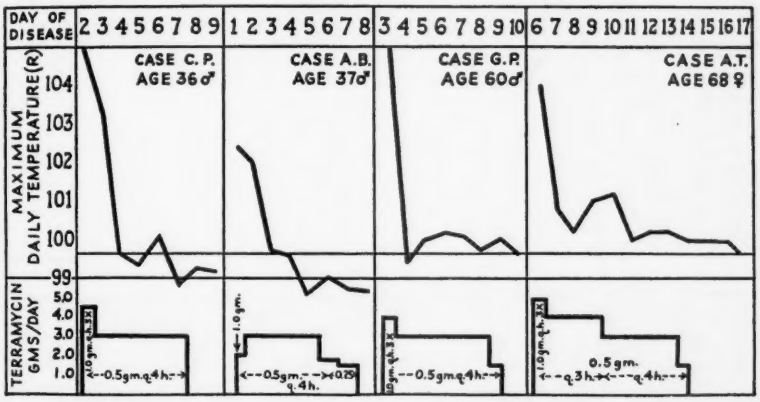

CHART 4. Cases C.P., A.B., G.P., and A.T. Pneumococcal lobar pneumonia.

from sputum sensitive to 1.0 μg./cc. of terramycin. Following an initial loading dose of terramycin of 1.0 gm. q.h.3x, he was treated through the 7th day with 3.0 gm. per day. The temperature fell critically to normal within 36 hours; convalescence was uneventful.

Case A.B., age 37, male. Chart 4. Pneumococcal lobar pneumonia, right middle lobe. Admitted on the 1st day of disease, moderately ill, blood culture negative. Pneumococcus from sputum sensitive to 0.5 μg./cc. of terramycin. Following an initial dose of 1.0 gm. of terramycin, he was treated with 3.0 gm. per day for 4 days and then with 1.5 gm. per day for 2 days. The temperature fell critically to normal within 24 hours, the signs of pneumonia resolved rapidly; convalescence was uneventful.

Case G.P., age 60, male. Chart 4. Pneumococcal lobar pneumonia, right upper, right middle, and left lower lobes. Admitted on the 3rd day of disease, acutely ill, auricular flutter, blood culture negative. Pneumococcus from sputum sensitive to 0.5 μg./cc. of terramycin. Following an initial loading dose of terramycin, 1.0 gm. q.h.3x, he was

treated with 3.0 gm. per day until the 9th day. The temperature fell from 105° F. to normal within 18 hours; recovery was prompt and uneventful.

Case A.T., age 68, female. Chart 4. Pneumococcal lobar pneumonia, right middle, right lower, and left lower lobes. Admitted on the 6th day of disease, acutely ill, blood culture negative. Pneumococcus from sputum sensitive to 0.5 μg./cc. of terramycin. Treatment with terramycin consisted of a loading dose of 1.0 gm. q.h.3x, followed by 4.0 gm. per day for 3½ days and then 3.0 gm. per day for 4½ days. There was prompt clinical improvement with sharp reduction in temperature but low grade fever

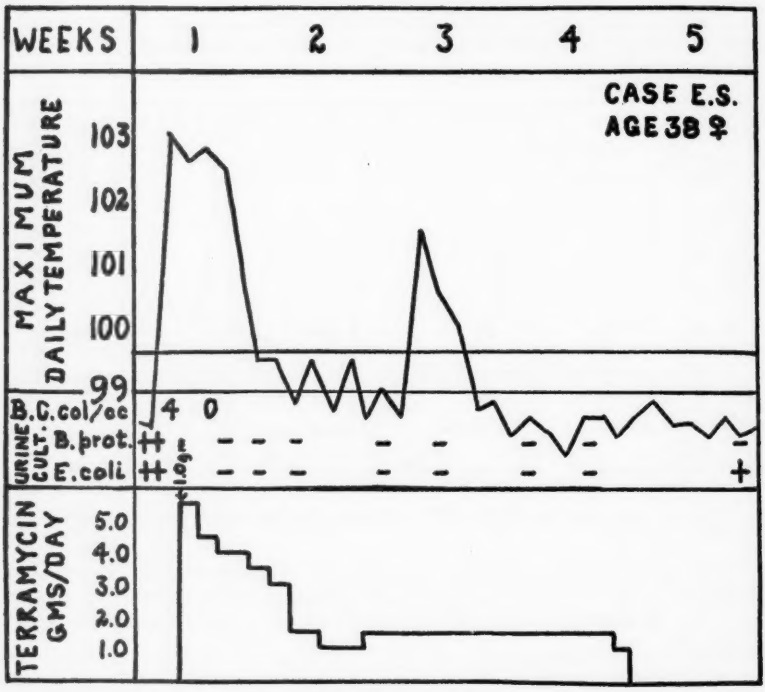

CHART 5. Case E.S. Urinary tract infection, *Streptococcus viridans* bacteremia.

for several days, a phenomenon often encountered in old people treated late in the disease with penicillin. Further convalescence was uneventful.

Case E.S., age 38, female. Chart 5. Subacute urinary tract infection with *E. coli* and *B. proteus vulgaris*, acute *Streptococcus viridans* bacteremia. This patient with transverse myelitis, paraplegia, and a nonfunctioning urinary bladder had had an afebrile subacute urinary tract infection for approximately one month, which had failed to

respond to treatment with sulfadiazine and streptomycin. While under treatment she had a shaking chill and sharp rise in temperature to 103° F. Blood culture showed 4 colonies per cc. of *Streptococcus viridans*. The sensitivity to terramycin of the *S. viridans* was 1.0 μg./cc., of the *B. proteus vulgaris* from her urine > 2.0 < 4.0 μg./cc. Terramycin treatment was promptly instituted with an initial dose of 1.0 gm. followed by 0.5 gm. q.2 h.12x and subsequent treatment for 3½ weeks as shown in Chart 5. The blood culture was sterile in 24 hours, and both *E. coli* and *B. proteus vulgaris* had disappeared from the urine cultures in 48 hours. The temperature was normal within 96 hours and remained so except for a transient unexplained bout of fever during the third week. As might have been expected, *E. coli* reappeared in the urine one week after treatment was discontinued.

Case C.S., age 72, male. Urinary tract infection with *E. coli* and *B. proteus*. *E. coli* sensitive to 4.0 μg./cc. of terramycin, *B. proteus* not tested. This patient with diabetes mellitus and arteriosclerotic heart disease in congestive failure had developed a perineal fistula and severe urinary tract infection following a prostatectomy. The infection had not yielded to treatment with sulfadiazine, penicillin, streptomycin, or chloromycetin. The effect of terramycin on the urinary tract infection was as follows:

Day	Terramycin gms./day	Urine cultures *E. coli*	*B. proteus*
1	...	++	++
2	...		
3	2.5		
4	3.0	±	—
5–7	3.0	—	—
8–12	1.5	—	—
13	...	—	—

Case E.D., age 72, female. Urinary tract infection with *B. mucosus capsulatus*. This patient with generalized arteriosclerosis, diabetes mellitus, and renal calculus had suffered approximately six months with a chronic cystitis which had failed to respond to sulfadiazine, penicillin, and streptomycin. Two weeks before admission she had developed an acute exacerbation. Cultures of the urine showed it to be heavily infected with *B. mucosus capsulatus*, which was found to be sensitive to 5.0 μg./cc. of terramycin, to < 25.0 > 5.0 μg./cc. of streptomycin. After an initial dose of 1.0 gm. of terramycin, she was treated with 0.4 gm. q.4 h. for 4 days followed by 0.25 gm. q.4 h. for 2 days. The symptoms of cystitis rapidly subsided. Urine cultures were sterile on the 3rd day and remained so until discharge on the 11th day.

Case J.G., age 46, male. Urinary tract infection with *B. mucosus capsulatus*. This patient, severely ill with hypertensive cardiovascular disease, diabetes mellitus, and nephritis with azotemia, had had symptoms of cystitis for approximately one month. On admission, cultures of the urine showed a heavy growth of *B. mucosus capsulatus*, which was found to be sensitive to 4.0 μg./cc. of terramycin. After 4 days of penicillin therapy without effect, he was treated with terramycin—initial dose 1.0 gm., then 0.5 gm. q.4 h. for 10 days. During the period of treatment there was considerable reduction in the albuminuria, pyuria, hematuria, and bacilluria, but the infection was not eliminated. Nausea and vomiting became quite troublesome, and the N.P.N. rose

from 85 mg.% to 117 mg.%. It seemed possible under these conditions that the excretion of terramycin in the urine might be inadequate to exert a bacteriostatic effect. Consequently, on the 9th day of treatment, the concentration of terramycin was determined in 4 samples of urine collected at 4:00, 5:00, 7:00, and 8:00 p.m., 0.5 gm. of terramycin having been given at 4:00 p.m. In all 4 samples the concentration was found to be 32 μg./cc., a considerably smaller amount than would be expected with normally functioning kidneys,[4] but still 8 times the concentration required to inhibit the growth of the patient's strain of *B. mucosus capsulatus*. Subsequent treatment with streptomycin also failed to eliminate the urinary tract infection in this patient.

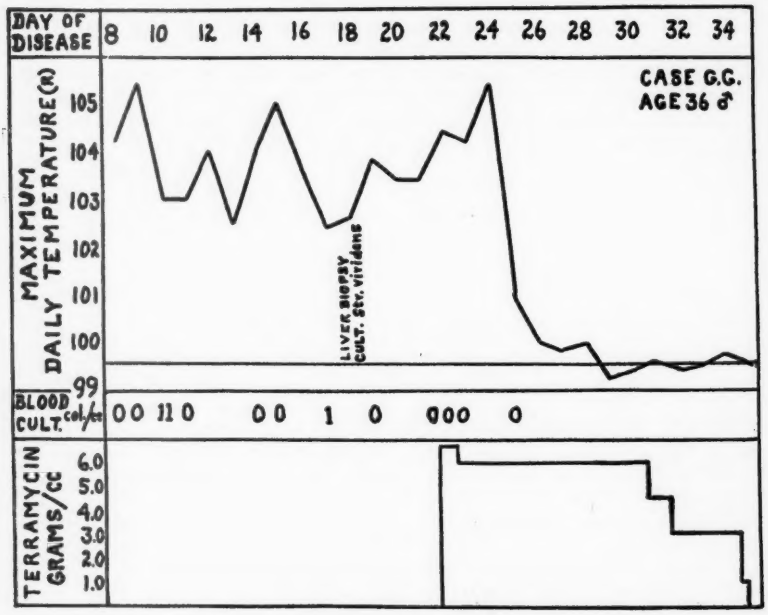

CHART 6. Case G.G. Cholangeitis lenta due to *Streptococcus viridans*.

Case A.P., age 56, female. Staphylococcal pyoderma. This patient with a diffuse, exfoliative dermatitis covering her face, trunk, and extremities, developed a severe bullous pyoderma of her fingers, palms, feet, and left lower leg and was admitted at once. By the 4th day she was critically ill with spreading pyoderma and a temperature of 102.8° F. Cultures of pus withdrawn from an unruptured bulla showed a heavy growth of *Staphylococcus aureus* and a moderate growth of *Staphylococcus albus*. Blood culture was negative. The *Staph. aureus* was sensitive to 1.0 μg./cc. of terramycin, the *Staph. albus* to 0.5 μg./cc. Treatment with terramycin was initiated on

the 4th day with 1.0 gm. q.h. 3x, followed by 0.5 gm. q.3 h. for 7 days and 0.25 gm. q.4 h. for 1 day. The temperature fell rapidly and reached normal within a week; the pyoderma subsided progressively and had disappeared by the 3rd day of treatment.

Case J.A., age 27, male. Buccal and penile fusospirochetosis. This patient, admitted on the 7th day of illness, presented the picture of a severe ulcerative Vincent's infection of the whole buccal mucosa and the glans penis, superimposed upon a preceding vesiculopapular eruption which involved the skin of the face, arms, and trunk, and the buccal mucous membranes. Smears from the gums and glans penis showed innumerable fusiform bacilli and Vincent's spirochetes. After 3 days without improvement he was treated with terramycin as follows: initial dose 1.0 gm. q.h. 3x, 0.5 gm. q.3 h. for 4 days, 0.25 gm. q.4 h. for 1 day. Within 24 hours fusiform bacilli and spirochetes were no longer demonstrable in smears from the gums and only a few spirochetes were seen in the smear from the glans penis. The lesions of the buccal mucosa and glans penis subsided rapidly and were healed by the 6th day of treatment.

Case R.S., age 53, male. Influenza. Admitted on the 4th day of disease, acutely ill, temperature 103.4° F. Terramycin, initial dose 1.0 gm. q.h. 3x, followed by 4.0 gm. per day for 2½ days had no apparent effect on the course of the disease.

Case G.G., age 36, male. Chart 6. Cholangeitis lenta. This patient was admitted on April 1, 1950, with a history of chills, fever, and general malaise for 8 days, which developed suddenly 9 days after a transient attack of mid-abdominal pain. Physical examination revealed only a slight icterus. As shown in Chart 6, he continued to run a high fever, and on 2 occasions blood cultures were positive for *Streptococcus viridans*. The diagnosis of cholangeitis lenta was finally established by a liver puncture biopsy which showed diffuse cholangeitis and pericholangeitis. Cultures of the liver tissue were positive for *Streptococcus viridans* similar to that obtained in blood cultures and also showed a non-hemolytic gamma streptococcus. The *Streptococcus viridans* was sensitive to 2.0 μg./cc. of terramycin. Terramycin therapy was instituted on the 22nd day with an initial dose of 1.0 gm. q.h. 3x, followed by 6.0 gm. per day for 8½ days. It was then reduced to 3.0 gm. per day for 4½ days. Within 96 hours the patient had shown marked improvement and the jaundice was diminishing rapidly. He appeared fully recovered by the 7th day of treatment, and further convalescence was uneventful.

Discussion

The number of cases described in this preliminary report is too limited and the variety of clinical material too great to warrant more than tentative suggestions concerning some of the infections in which terramycin should prove to be an effective antibiotic agent. Among these are pneumococcal lobar pneumonia; staphylococcal infections, both focal and generalized; alpha hemolytic streptococcal infections, including both *S. viridans* and *S. fecalis;* urinary tract infections with gram-negative bacilli, including some strains at least of *E. coli, B. mucosus capsulatus,* and *B. proteus;* and

Vincent's fusospirochetosis. The apparent cure of Case E.S. with staphylococcal endocarditis, of Case G.G. with severe diffuse intrahepatic cholangeitis, and the prompt recovery of three cases of lobar pneumonia treated early in the disease lend strong support to these suggestions.

In the two cases of subacute bacterial endocarditis the response to terramycin appeared to be essentially the same as might have been expected with adequate doses of penicillin for an equivalent length of time. Shortly after terramycin was stopped, however, relapse occurred in both cases. Whether more prolonged treatment would have been successful is uncertain at present.

The four cases of urinary tract infection were selected to determine whether infections which had not yielded to other chemotherapeutic and antibiotic agents would respond to terramycin. While this occurred promptly in three of the four cases, it is of course likely that reinfection of the urinary tract in these cases will recur, and in fact it had done so in one case (E.S.) within a week after treatment had been discontinued.

The dosage employed was somewhat arbitrary and may have been more than necessary in some cases, particularly with respect to the initial or loading dose. There is a suggestion, at least, that a single dose of 1.0 gm. instead of three doses at hourly intervals would often be sufficient. Much further study will be required to define the most appropriate dosage for various infections. Equally uncertain at present is the minimum duration of treatment that will be effective in preventing relapse.

The only untoward side effects observed were nausea, vomiting, and frequent loose stools. As will be seen from Table 1, 7 cases had no nausea or vomiting, 10 cases no vomiting, and 6 cases no frequency of stools. In only 2 cases (W.P. when receiving 6.0 gm. daily and J.G. with nephritis and azotemia) were nausea and vomiting troublesome. In 7 of the 8 cases with loose frequent stools, this phenomenon occurred for 2 to 3 days with 2 to 4 stools per day. In only 1 case (C.S. with perineal fistula) was it troublesome or persistent.

Summary

The results of terramycin therapy in 15 patients with various infections have been described. The response to therapy in 12 was sufficiently prompt and clear cut to warrant the suggestion that terramycin will prove to be a valuable antibiotic agent for the treatment of a considerable variety of human infections caused by terramycin-sensitive microorganisms.

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SOME REMINISCENCES OF THE "PATHOLOGICAL" IN THE EARLY DAYS OF THE JOHNS HOPKINS HOSPITAL AND MEDICAL SCHOOL

GEORGE BLUMER

In 1889 when I began the study of medicine, many medical students still had preceptors. I was fortunate to have as my "guide, philosopher and friend," Dr. Charles Pemberton Murray, a graduate of Princeton and of the College of Physicians and Surgeons in New York. When he heard that I was to study medicine, he suggested that I ride over to his house, a couple of miles east of Sierra Madre where I lived, and he would quiz me twice a week on Gray's *Anatomy* and Dalton's *Physiology.* In the vacation between my first and second years he quizzed me on Austin Flint's *Practice of Medicine,* the pathological sections of which had been written by William Henry Welch. I can distinctly recall that on one occasion Dr. Murray advised me that if I ever got a chance to study under William H. Welch, I should not fail to grasp it.

I graduated in December, 1891, and at the end of 1892, having served my first interneship at the old City and County Hospital in San Francisco, I was at a loose end, for I was still not old enough to get a license to practise medicine. My cousin, Dr. G. Alder Blumer, to whom my father turned for advice, was a friend of Dr. Henry M. Hurd, the first superintendent of the Johns Hopkins Hospital, and he suggested that I go to Baltimore early in 1893 and take some of the graduate courses which were then offered there. I therefore entered the classes in pathology and bacteriology, subjects which, except for a little gross pathology, had not been included in my medical school training. Pathology was taught by Dr. Welch himself, by his assistant Simon Flexner, or by the Fellow in Pathology, Lewellys F. Barker. Bacteriology was taught by George F. Nuttall, until he left to occupy a chair in Cambridge University, and after that by B. Meade Bolton.

The pathological laboratory commonly called merely the "Pathological," was in the extreme northeast corner of the Hopkins Hospital lot at Monument and Wolfe streets, just opposite Hanselman's saloon, which was a "hangout" in times of leisure for the staff and later for medical students. The "Pathological" was a three-story brick building, the first and second

floors occupied by bacteriology and pathology, the third by pharmacology.

My first experiences in the building were as a graduate student. Bacteriology was taught in a large classroom on the first floor equipped with the necessary work benches, Bunsen burners, sterilizers, saucepans, and material for making media. Each student was required to make his own media and when this had been sterilized and transferred to tubes, flasks, or petri dishes, he was given cultures first of non-pathogenic and later of the common pathogenic microörganisms which he had to grow and examine after staining with aniline dyes, including Gram's stain. Students were also taught how to inoculate laboratory animals, particularly rabbits and guinea pigs, and how to examine them after death in case they succumbed.

Gross pathology was taught in the autopsy room and microscopic pathology in a room on the second floor corresponding in location with that used for bacteriology on the first floor. I can recall but few of my fellow students except the lanky, sandy-haired doctor who always sat at my left in the classes on pathological histology. I got into conversation with him the first day of the session and immediately discovered that he was English. His name, he told me, was James Carroll and he was a medical officer in the United States Army. He and I became quite well acquainted and I saw him from time to time until his death a few years after his yellow fever experiences.

Each of the sessions in pathological histology was preceded by an informal talk either by Dr. Welch himself or by Simon Flexner or Lewellys Barker. Then sections were given out which we stained with hematoxylin and eosin and studied, the instructors acting as demonstrators. At the completion of the course, unknown sections were distributed and we were required to write out a description of each one and record our diagnosis. Our papers were later returned with comments, and for years I kept mine with remarks written on it in Dr. Welch's characteristic script.

In May of 1893 I joined Halsted's staff and one of my duties consisted in assisting the Resident in Surgery, Joe Bloodgood, commonly nicknamed "Bloodclot," in the examination of specimens removed at operation. We had a room in the "Pathological," and spent several hours almost every afternoon in recording gross descriptions of the surgical material, placing selected pieces in hardening fluid, and later, after section and staining, adding the histological diagnoses to the records. Joe Bloodgood was intensely interested in this work, but as Resident was constantly being called away to the wards or admitting office so that most of it fell to me.

After a year under "the Chief" in charge of medical bacteriology, I joined Dr. Welch's staff in the spring of 1895 as Assistant in Pathology. I still lived in the hospital, but my work was all in the "Pathological," demonstrating in pathology and bacteriology to second- and third-year medical students and to graduate students. To the latter I had occasionally to give preliminary talks in bacteriology. Other duties were: making autopsies, preparing sections for medical and graduate students, and examining and reporting to Dr. Welch on specimens from outside the hospital which were sent to him for his opinion. One New York gynecologist, Dr. Gill Wylie, continued to send most of his operative material to Dr. Welch long after the latter had left the metropolis.

Some incidents of unusual interest to me occurred during the course of the sixteen months that I spent under "Popsy," who was always ready to give help and advice when he was in the laboratory. (The same was true of Simon Flexner who was in charge when Dr. Welch was absent.) One day, while studying a section from tissue labeled epithelioma that I had picked out for classroom use, I ran across a histological picture which completely puzzled me as it was unlike any epithelioma I had ever seen. I took the section to Dr. Welch who examined it and said, "That is a section of sponge." He reminded me that before the days of modern skin-grafting and plastic surgery, large defects, left after removal of a malignant tumor, were often filled in by the surgeon with a slice of ordinary sea sponge which acted as a scaffolding for new tissue formation and shortened the period of healing.

On another occasion Dr. Welch called me into his room to show me a section that he had been studying for a week. "He had to decide," he said, "whether it was sarcoma or granulation tissue." He finally concluded that it was granulation tissue mainly on account of the parallel arrangement of the blood vessels which were roughly at right angles to the surface. He told me confidentially that the decision was very important because the specimen was from the thigh of President Grover Cleveland. Several years previous Cleveland had been secretly operated on for a sarcoma of the jaw. The matter is no longer secret for a description of the incident, the operation being performed on a ferry boat in Long Island Sound, came out several years ago in the *Saturday Evening Post*. The operation was performed by Dr. Joseph Bryant of New York, an old friend of Dr. Welch, and a skillful dentist in the party made a temporary denture which concealed the fact that the President had been operated on, and that part of his jaw had been removed.

During my service with Dr. Welch the location of the pharmacological laboratory on the top floor became, at one period, significant. This was when "Bunny" Aldrich was working on the chemistry of the secretion of the anal glands of *Mephitis mephitica,* commonly known as the skunk. There were periods when, as Eugene Field remarked in his poem "When Willie Wet the Bed," "Sabean odors clogged the air." Aldrich's clothing became so imbued with the stench that he was unable to get rid of it. He had to ride home in a streetcar, and it was creditably reported that he usually occupied one corner of the vehicle while the rest of the passengers crowded into the other end—as far away from him as possible.

There were several situations about the pathology of those days that would amuse a modern pathologist. There was the traditional story of "Counce" (W. T. Councilman who had gone to Harvard before my advent), riding out to Bayview to do autopsies. His tricycle had affixed to it a specimen box which he filled with removed viscera and which is said to have leaked blood and other juices all the way back from Bayview to the "Pathological." The autopsies were all recorded in longhand in books and many are the ones I have written as an interne as Dr. Welch, or more commonly Dr. Flexner, dictated it in the course of the autopsy. As a matter of fact, I learned the order of procedure in making autopsies by this recording. I cannot recall that anyone ever gave me formal lessons in the art of the dead house. The methods of that day were less complicated than at present. Specimens were usually hardened in alcohol, occasionally in Müller's fluid. They were usually embedded in celloidin. We generally cut and stained our own sections, and hematoxylin and eosin were almost the only stains used except for the central nervous system. It was common practice to make frozen sections of organs like the heart, liver, or kidney whose cells might show fatty changes which the hardening agents would remove. Cultures were routinely taken at autopsies from the heart's blood and most of the organs. The staining methods later developed by Van Gieson and Mallory were not then generally in use, and the use of formaldehyde as a hardening agent and for preserving gross specimens in natural colors was in its infancy; but it was an intellectually vivifying experience to be brought into daily contact with men like Drs. Welch and Flexner and with the alert-minded students which the new style Hopkins' Medical School attracted.

THE GIANT-CELLS OF MEASLES*

C. H. BUNTING

The histological study of the tissues of those infected with the virus of measles has shown a variety of structures perhaps not improperly called giant-cells. The most striking of these are the masses of fused epithelial cells in the air-passages and the cells occurring chiefly in superficial and deep lymphoid tissue. Although the latter, which are of the greater interest, were described by Alagna[1] in 1911, they appear to have escaped any general attention until after the publications of Warthin[22] and of Finkeldey[10] twenty years later. Warthin described the giant-cells in the tonsils removed surgically from four patients in the prodromal or pre-eruptive stage of the disease, while Finkeldey described them in the tonsils of one case and later in the appendix of another.

Since that time, published cases of their occurrence in surgical and post-mortem tissues have been numerous. Elizabeth Corbett[8] has tabulated the cases occurring to the date of her publication, 1945, and finds 12 cases (to which may be added 4 overlooked by her) in which the cells were found in the tonsils, and 13 cases with the cells in the appendix. In post-mortem material (including the one which she reports), in addition to those locations, they were found in lymph nodes, spleen, thymus, Peyer's patches, and the respiratory mucous membranes. It is to be noted that the records of the post-mortem cases show a marked irregularity of distribution of the giant-cells in the various lymphoid tissues. To this report may be added the experimental production by Gordon and Knighton[12] of the same or similar cells in three of four monkeys which were injected with the blood of measles patients.

From the records it is evident that the formation of these cells takes place in the prodromal or pre-eruptive period of the disease. They have been found from four days before the appearance of the rash to the time of its occurrence and in one case, that of Masugi and Minami,[15] even four days afterward. This period of occurrence is corroborated by the failure of Degen[7] to find them in his large series of cases that died late in the disease from pyogenic complications.

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The question as to whether the cells are specific for measles has been decided in the affirmative by most authors. An exception is Tomlinson,[21] who examined the tonsils of a hospital patient, a five-year-old girl, and gave the clinicians a probable diagnosis of measles upon finding the giant-cells in the tonsils, only to have her develop chicken pox to which she had been exposed in the ward a suitable time before. Those who believe in the pathognomonic character of the lesion are inclined to doubt the diagnosis of chicken pox, in my opinion unjustifiably.

The descriptions by the various authors and their published photographs show definitely that, with the exception of Mulligan,[17] they are dealing with the same abnormal cell-form. (Mulligan depicts relatively small giant-cells with large, vesicular, lobed or multiple nuclei, with large nucleoli, which he designates lymphoid stem-cells. Such cells are not confined to the prodromal stage of measles but may be found throughout its course and may closely approximate in character the typical giant-cells of Hodgkin's disease.)

The descriptions by the various authors in general parallel that of Warthin[22] who described the cells as "round or irregularly lobed. . . . The nuclei were arranged in a grape-like cluster in the center of the cell. They were granular and stained lightly with haematoxylin. . . . The giant cells varied greatly in size and in number of nuclei, many of them averaging about 100 microns. There were some with as many as . . . 70 to 100 nuclei. The largest had a rather scanty cytoplasm that stained bright red with eosin. In these large forms the nuclei were often pyknotic and fragmented. . . . All possible transition stages of development from the hyperchromatic lymphocyte form to the largest multinucleate giant-cell were present."

To Warthin's description, Finkeldey,[10] in discussing the fate of the cells, has added that "there follows a pyknosis and chromatolysis of the nuclei, and the chromatin masses flow together into a homogeneous blue-black-colored mass." In a further change these nuclear masses break up and dissolve into small spherical droplets. Such pictures, hinted at by Ciacco,[4] occur in the illustrations of numerous articles (Hathaway,[13] Corbett,[5] Davidsohn and Mora,[6] *et al.*). While these massive giant-cells with many nuclei appear to predominate in most cases, in the sections of his case, Schultze[18] found but one such, and in the other cells the average of nuclei to a cell was but ten.

Speculation as to the nature and the pathogenesis of these giant-cells has been limited and has led to no generally accepted conclusion. Warthin[22] assumed that it was a "defense cell" formed by "amitotic division of nuclei in hyperchromatic cells resembling lymphocytes." Finkeldey[9] suggests that

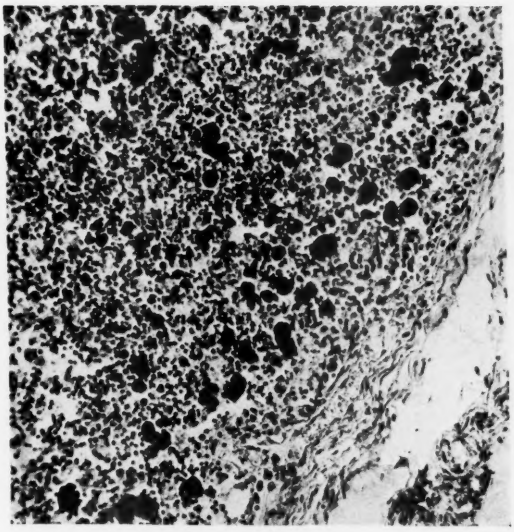

FIG. 1. Agglutinated lymphocyte nuclei in depleted cortex of thymic lobule. H & E stain. x 225.

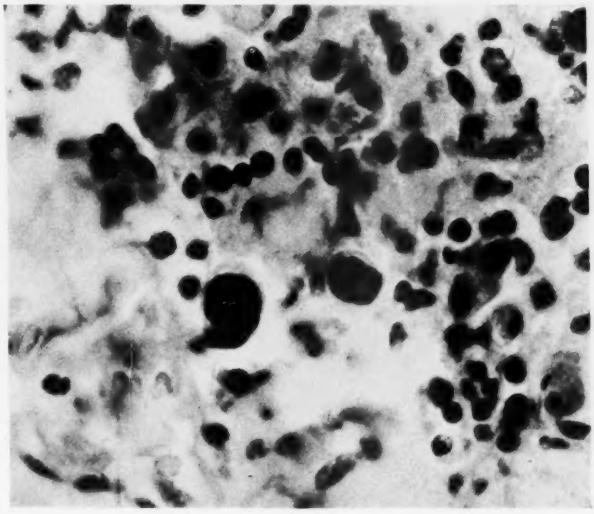

FIG. 2. Two phagocytes containing agglutinated lymphocyte nuclei. H & E stain. x 1100.

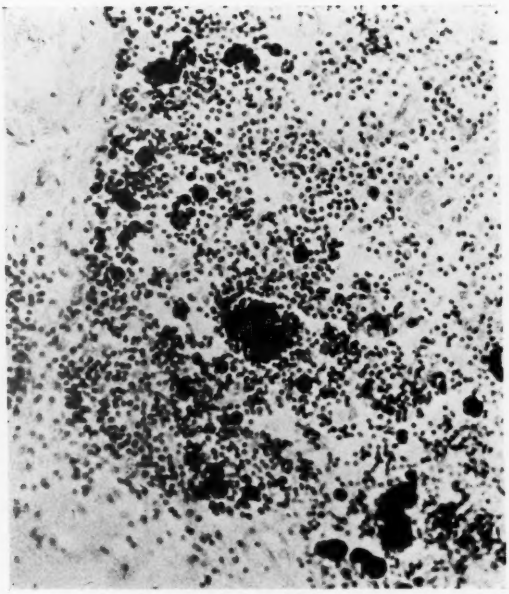

FIG. 3. Massive agglutination of lymphocytes without phagocytosis. H & E stain. x 280.

"while one can assume that there is an excessive nuclear proliferation following an abnormal stimulus, one can also find pictures that lead one to think of cell-fusion." He also suggests the possibility of an allergic reaction to an endogenous toxic stimulus. Mayerhofer[16] seconds the suggestion of an allergic phenomenon.

Wegelin[25] notes that the nuclei resemble those of reticular cells but are smaller, and suggests that the rapid nuclear proliferation leads to a reduced size of the daughter nuclei. He gained the impression that in the mucosa of the appendix the giant-cells are derived from lymphoblasts or lymphocytes, since in these there was a greater number of small dark nuclei. Schultze[18] and Fischer[11] agree that the nuclei are of the size of small lymphocytes.

Hathaway[13] in discussing his case, in which there was a general dissemination of giant-cells, concludes that they arise from reticuloendothelial cells of lymphoid sinuses in the glands and from those of the splenic pulp. He notes also that the giant-cells showed considerable phagocytosis for leukocytes.

Herzberg[14] is of the opinion that the giant-cells are "made up apparently of agglutinating or coalescing cells of lymphoid origin." In some instances these groups of nuclei lacked a surrounding protoplasm, while in others it was distinct and stained quite deeply with eosin. Herzberg considered that his observations confirmed Warthin's[22] suggestion of a distinct pathological change in the lymphatic structures in the prodromal stage of measles. Corbett[5] concluded that a fusion of lymphocytes is the most likely explanation of the giant-cells "although the large number of plasma cells found in the tissue surrounding the giant-cells leads one to suspect that they might be implicated in giant-cell formation."

In view of the evidence afforded by the following case in regard to the pathogenesis of the lymphoid lesions, it seemed worthy of report. It was the only case to fall in this interesting prodromal period among the cases of measles which had come to post-mortem examination in the service of Dr. Winternitz at the Yale Medical School and was encountered in a review of the histological sections of the cases in a study of the lymphoid reactions in the disease.

The patient was a white girl, 10 months of age, who had been admitted to the New Haven Hospital the day before her death with a history of having been exposed to measles in a brother and having been seized with vomiting three days before, when her temperature was 102.4. Vomiting, apparently with abdominal pain, continued irregularly on the days subsequent to admission and on February 29, 1928, her temperature rose to 106. She had some cough.

On admission she appeared to be a desperately sick child, dehydrated, somewhat cyanotic and comatose with a temperature of 106.3. She showed late Koplik spots and a very early rash on the back of the neck. There was no chest dulness but rales were heard in the left interscapular region. The ear drums were retracted and there was a conjunctivitis. Rbc—4,000,000; Wbc—4,000; neutrophils—64 per cent.

The patient died at 5 a.m., March 1, 1928, and came to post-mortem examination five hours later.

Clinical diagnosis: severe measles, otitis media, bronchopneumonia.

Anatomical diagnosis: acute tracheobronchitis, pulmonary congestion, and oedema.

The gross and histological findings, except for those to be described, were inconspicuous. As this post-mortem examination was held before the descriptions by Warthin[23] and by Finkeldey[9] of the lesions in tonsils and appendix, tissues from these organs were not saved for section. This is particularly unfortunate in regard to the latter organ since, clinically, the case suggests one of measles-appendicitis.

Of the organs, the thymus offered the most striking histological picture which on first glance justified Corbett's[5] description of the organ in her case as showing "a multitude of giant-cells, so numerous, so dense and hyperchromatic as at first to suggest an artefact." Such hyperchromatic masses of various shapes and sizes are scattered irregularly throughout the section but confined almost exclusively to the cortical zone of the organ. In some lobules this zone is apparently entirely depleted of lymphocytes and resembles the medulla in architecture; in other lobules, there is but partial depletion, while still others have a normal lymphoid cortex. The number of giant-cells is roughly correlated with the degree of disappearance of lymphocytes from the cortex. These so-called giant-cells are generally in the state first described by Finkeldey in which the nuclei are fused into irregular hyperchromatic masses. With very light staining with haematoxylin, one can still distinguish in these fused masses the outlines of the nuclei of which they are composed. In other cells, however, the nuclei, in number from few to many, are still discrete, usually pyknotic and fragmented. Of these masses, by no means all or possibly even a majority show a cellular membrane about them. Extracellular agglutinated lymphocyte nuclei, from two or three in the plane of section to many, are numerous. Many of these masses are so large that phagocytosis by a single cell would not be possible. Some of this type show a "foreign body reaction" in the gathering of phagocytic cells about them, although actual fusion of these cells was not observed. In the cells with protoplasmic border, if the plane of section is favorable and the cell not too crowded with fused nuclei, there is discernible an eccentric, vesicular nucleus crowded against the cell membrane, obviously the proper cell-nucleus.

The perilaryngeal and peribronchial lymph nodes show some agglutination of lymphocytes, and a few macrophages with ingested lymphocytes.

In the trachea, the major bronchi, and the intrapulmonary bronchi, which are lined by pseudo-stratified columnar epithelium, are seen numerous groups of epithelial cells with deeply staining, pyknotic nuclei, which have lost their cell boundaries and are fused into giant-cell-like masses. Some of these have been desquamated and lie in the lumina of the bronchi, leaving a denuded bronchial wall.

In the lung in addition to congestion and oedema, one finds in the capillaries not only many megalokaryocyte nuclei but also numerous deeply haemetoxylin-staining lobulated masses, which, from the size of the component lobules, are evidently made up of agglutinated lymphocyte nuclei. These appear to be adhesive as well as cohesive, as in some vessels they appear affixed to the wall without blocking the vessel lumen or stopping blood flow in it.

Comment

One might expect, a priori, that in this prodromal period of measles he would find evidence of injury and not of reaction. These observations show definitely that there is such injury to at least respiratory epithelium and to lymphocytes in the lymphoid tissues, leading to the abnormal cell-forms under discussion. It seems reasonable to accept the findings of Broadhurst[8] and his associates in smears from Koplik spots—that the presence of inclusion bodies is accompanied by erosion and disintegration of the cytoplasm of host cells, both epithelial and lymphocytic. This apparently begins as a peripheral cytolysis leading early to a ragged, filmy periphery. Such a change would account for the loss of cell borders and fusion of the epithelial cells into the large giant-cell-like masses seen in various stages of desquamation from the bronchial walls.

In the lymphoid tissue the small lymphocytes are attacked by the virus. The cells lose their cytoplasm and the nuclei are phagocytosed in large numbers by the macrophages, chiefly the reticuloendothelial cells, which thus constitute the characteristic giant-cells. With these phagocytes the nuclei behave entirely differently from the lymph cells in macrophages in the thymus during physiological atrophy of the gland. There is no rapid digestion but instead, the nuclei fuse into the hyperchromatic masses described. Similar masses but extracellular are formed by agglutination of lymphocyte nuclei.

This destruction of lymphocytes clearly explains the depletion of the thymic cortex. With a similar process in other lymphoid structures one may account for the circulatory lymphopenia occurring early in the disease. As

Bunting and Thewlis[2] have shown, this is in proportion to the severity of the attack. In severe cases, the lymphocytes may be fewer than 400 per cu. mm. (from a normal average of about 1800 cu. mm.) at the beginning of the rash. The similar lymphopenia which occurs in chicken pox offers only a very mild suggestion that the diagnosis was correct in Tomlinson's[21] case and that the phagocytic giant-cell is not specific for measles.

Somewhat enlarged reticuloendothelial cells with lobed or multiple large nuclei, as found by Mulligan[17] and by Fischer,[11] are not confined to the prodromal period of the disease but may be found throughout its course, as I have noted.

The conclusion to which this study has led is that the "giant-cell" of lymphoid tissue in measles is a macrophage loaded with the nuclei of lymphocytes which have been attacked by the virus.

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INTERSTITIAL DESOXYRIBONUCLEIC ACID FOLLOWING CELL DEATH*

HENRY BUNTING

In regions of necrosis there are commonly seen in histological preparations hematoxylin-stained areas in the interstitium. These sometimes surround cells of the exudate which may be disintegrating, or they may be found immediately around the necrotic parenchymal cells of the particular organ. The nature of these areas for which hematoxylin has such a strong affinity has been investigated by several histochemical procedures.

Methods

Tissues were fixed in 10% formalin, Zenker's fluid, and Helley's fluid. They were dehydrated in the usual manner and imbedded in paraffin. Hematoxylin and eosin preparations were made on all. Progressive staining for several hours with dilute methylene blue, 10^{-4}M, in phosphate-sodium hydroxide buffer at pH 6.6, followed by drying in air and clearing in toluol, was used to determine the locations of basophilia. Such sections served as controls for those exposed to ribonuclease for the identification of ribonucleic acids. Sections were incubated for 2 hours at 58-60°C. in a solution of the enzyme† (6 mg. enzyme per 20 ml. of distilled water). Progressive staining in a dilute solution of aniline blue, 5×10^{-5}M, in citric buffer at pH 5.2 for several hours was used to determine areas of acidophilia. Feulgen reactions for desoxyribonucleic acids were carried out by exposing sections to Schiff's reagent (fuchsin-sulfurous acid) after hydrolysis in 1N HCl at 60°C. for intervals of from 10 to 60 minutes. (For details of the method, cf. Lison[6] or Stowell.[8]) Control sections were treated similarly except that water was substituted for HCl. Of the various basic fuchsins used in the preparation of Schiff's reagent (pararosanilin, C.I. no. 676; the mixture known as basic fuchsin, C.I. no. 677; and "new fuchsin," the trimethyl fuchsin, C.I. no. 678[2]), "new fuchsin" gave the deepest color and hence appeared to be the most sensitive reagent.

Inorganic phosphorus was sought in those tissues that had been fixed in formalin (the mercuric chloride in Zenker's or Helley's made them unsuitable). The method which has been used with success in control sites has been adapted from that of Feigl[4]: hydrolysis has been carried out for 5 minutes with 0.5% HNO_3 in the presence of

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† Ribonuclease from Armour & Co., prepared by Kunitz' method and with the non-specific proteolytic activity destroyed as per M. R. McDonald (J. Gen. Physiol., 1948, *32*, 39).

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2.5 ammonium molybdate solution; benzidine and sodium acetate solutions have been used as in the original.

Results

Examples of necrosis showing areas of hematoxylin-stained interstitial material have been selected from different sites. Sixteen have been studied as follows: 9 of the myocardium, 3 in the pancreas, 2 in the kidney, and 2 of tuberculous exudate. The uniformity of the histochemical findings made it seem unnecessary to enlarge further this group before reporting the results. The data will be described in the categories listed above.

Myocardium. Surrounding the necrotic muscle in an infarct of the myocardium there is a zone of edema and inflammatory cells. Many of the polymorphonuclear leukocytes are necrotic with pyknotic and karyorrhectic nuclei. It is in this area that hematoxylin stains conspicuously the granular or stringy-appearing substance, apparently precipitated protein, which lies between the cells of the exudate and extends into the adjacent connective tissue septa (Fig. 1). The hematoxylin staining of the intercellular material is not found to extend for any distance either into the area of necrotic or that of the intact myocardium. Methylene blue (Fig. 2) and aniline blue stain the same intercellular material intensely, indicating the existence of both acid and basic groups. Digestion in ribonuclease does not alter the staining by methylene blue of this granular and fibrillar material, indicating that ribonucleic acids probably do not contribute to the basophilia. The material gives the Feulgen reaction (Fig. 3), allowing the conclusion that desoxyribonucleic acid is present; this is consistent with the affinity shown for hematoxylin and methylene blue. These same reactions as a rule are not given by the necrotic muscle fibers, although in rare instances they are, suggesting that the desoxyribonucleic acids have diffused from the nucleus but are still confined within the cell membrane.

Pancreas. Small foci of necrosis of the parenchyma and fat cells of the pancreas are not an uncommon finding at autopsy. The granular or fibrous material that occupies most of the necrotic area stains deeply with hematoxylin (Fig. 4). Around the margin, leukocytes and other cells are frequently pyknotic. Fat cells are altered and show a granular rim of cytoplasm; this also stains with hematoxylin. All the above loci are basophilic, staining readily with methylene blue; the intensity is not diminished by exposure to ribonuclease. There is also acidophilic material present, as it stains with aniline blue to about the same intensity as do nuclei. The Feulgen reaction is given strongly by nuclei and by the granular and fibrous appearing necrotic material (Fig. 5) and somewhat less

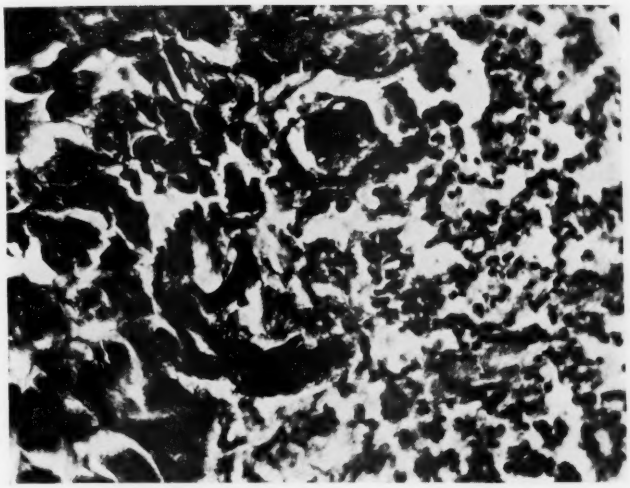

FIG. 1. Margin of myocardial infarct. Leukocytes and hematoxylin-stained precipitate in the interstitial region between the necrotic muscle fibers. Hematoxylin and eosin. x 380.

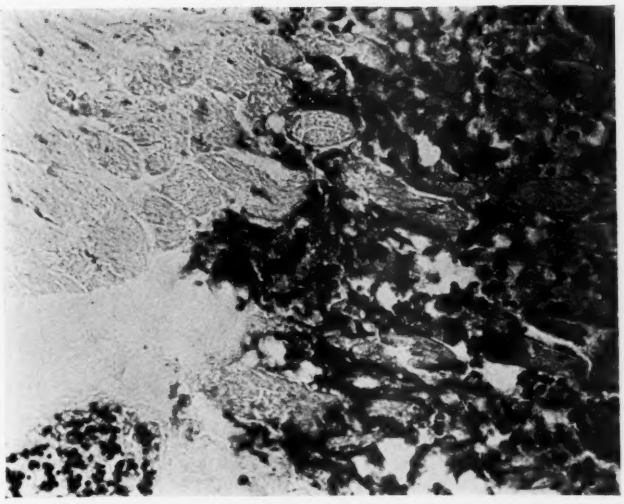

FIG. 2. Same field and magnification as preceding figure. Stained with methylene blue alone.

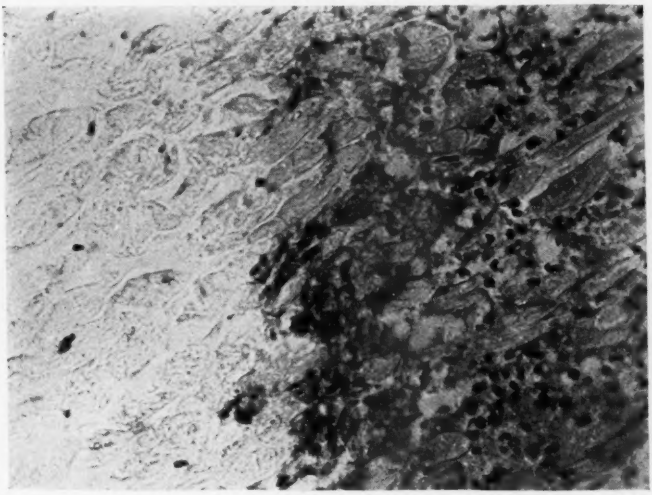

FIG. 3. Same field and magnification as preceding figures. Feulgen reaction, no counterstain.

FIG. 4. Focal area of necrosis in pancreas. Hematoxylin staining of necrotic center and granular margin containing cell debris; leukocytes and intact parenchyma beyond. Hematoxylin and eosin. x 380.

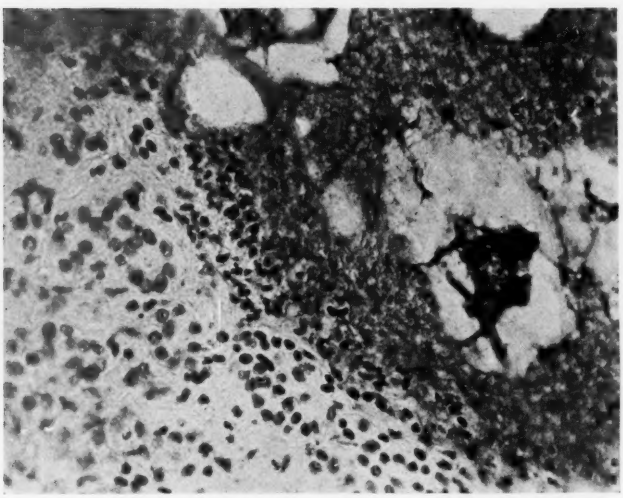

FIG. 5. Same field and magnification as preceding figure. Feulgen reaction, no counterstain.

intensely by the granular rim of the fat cells. It should be noted that the Feulgen reaction is absent in the basophilic material (which is also stained by hematoxylin) normally present in the basal portion of the cytoplasm of unaltered acinar cells or which may be found diffusely spread throughout their cytoplasm as a result of post-mortem autolytic changes; this basophilia being diminished or removed by treatment with ribonuclease is probably due to the presence of ribonucleic acids.

Kidney. Infarcts in the kidney give much the same picture as seen in the myocardium. At the margin is a band of intact and disintegrating polymorphonuclear leukocytes. The nuclear fragments are Feulgen positive and a faint reaction is also present in the intervening framework and necrotic epithelial structures. The cellular debris that is identifiable within the preëxisting tubular basement membranes also is strongly Feulgen positive. The material that gives the Feulgen reaction also stains with hematoxylin and is basophilic as judged by affinity for methylene blue.

Tuberculous exudate. Examples of tuberculous exudate have been chosen from the leptomeninges in tuberculous meningitis and a caseous lymph node. Pyknotic nuclei and karyorrhectic fragments are recognizable in the zone of necrosis; these as expected give the Feulgen reaction. The granular necrotic material, unrecognizable except as being cellular debris, in certain caseous areas stains faintly with hematoxylin and methylene blue; affinity for the latter is not affected by previous treatment with ribonuclease. Tests for inorganic phosphorus were negative. Aniline blue stains these areas conspicuously. Significantly, these basophilic regions give a Feulgen reaction. It may be of some interest that they may be separated for some distance from nuclear debris and also from the fibrous capsule around the caseous tissue.

Discussion

In the present studies various histochemical tests have been applied to extracellular areas which are often associated with necrosis and which characteristically show an affinity for hematoxylin. These regions were found to have reactive acidic groups as evidenced by their staining with the basic dye, methylene blue. This staining was not altered by ribonuclease as was that of certain intracellular sites of basophilia which were, therefore, concluded to be due to ribonucleic acid; these latter also showed an affinity for hematoxylin. While the basophilia of the extracellular areas, as determined by combination with methylene blue at a fairly high pH of 6.6, could be due to many kinds of acid radicals, it might be expected to be phosphoric acid. The Feulgen reaction presumably consists of a combination

between Schiff's reagent and the aldehyde groups of the desoxyribose molecules which have been freed from the nucleic acids by acid hydrolysis.[8] Since in all instances the regions under discussion gave a positive Feulgen reaction, they probably represent areas rich in desoxyribonucleic acid. Whether the nucleic acids are combined with the protein moity, the histones, cannot be stated. That the latter are present, however, is suggested by the strong affinity these areas have for the acidic dye, aniline blue, a reaction comparable in intensity to that shown by the nuclei themselves. The staining of chromatin by hematoxylin (actually hematein, the oxidized form in combination with a mordant) has, of course, been known for a great many years, but no chemical explanation has been given for the linkage other than the assumption that a lake is formed through the divalent cation constituting the mordant;[3] this reaction cannot be considered of histochemical significance.

The origin of the extracellular interstitial desoxyribonucleic acids is obvious from the close proximity of pyknotic and karyorrhectic nuclear fragments, whether of cells of the organ parenchyma or the exudate. It is suggested that the nucleic acids diffuse from the nucleus through the cell membrane upon the loss of its selective permeability or actual destruction associated with necrosis. In the interstitial regions the nucleic acids appear often to be associated with a granular precipitate undoubtedly derived from cell debris or the fluid components of the exudate. In some instances, such as infarcts, interstitial desoxyribonucleic acids are found only where cell death has been of fairly recent origin. Here it can be surmised that loss of nucleic acids from the area may occur by way of the lymphatics along with the edema fluid or that degradation beyond the level of recognition by these methods may result. Elsewhere, as in tuberculous caseation, detectable desoxyribonucleic acids remain for what must be relatively long periods of time, since they are found in the midst of caseous tissue; this is not wholly unexpected since tuberculous tissue is non-vascularized.

The changes described in the present studies are almost identical with those reported by Sherry, Tillett, and Christensen[7] of the occurrence of desoxyribonucleoprotein in pleural exudates. Feulgen stainable nuclear fragments and other material were present in the granular debris of the sediment. Klemperer[5] has identified Feulgen positive material as being responsible for the faint hematoxylin staining of the cytoplasm of certain necrotic cells characteristic of lupus erythematosus. All of these studies give a better understanding of certain regressive phenomena associated with cell death.

Summary

The hematoxylin-stained material commonly seen extracellularly in the interstitium of regions of necrosis, such as infarcts or caseous exudate, has been subjected to various histochemical tests; the material exhibits both basophilia and acidophilia, is not affected by ribonuclease, and gives the Feulgen reaction. The staining qualities are concluded to be consistent with the presence of desoxyribonucleic acids and associated histones derived from nuclei of necrotic cells of the exudate and the parenchyma.

Appreciation is expressed for the technical assistance of Miss Patricia Reynolds.

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THE MORPHOLOGY AND STAINING CHARACTERISTICS OF THE *TREPONEMA PALLIDUM*. REVIEW OF THE LITERATURE AND DESCRIPTION OF A NEW TECHNIQUE FOR STAINING THE ORGANISM IN TISSUES*

ROBERT E. CAMPBELL AND PAUL D. ROSAHN

In 1905, sixty-eight years after Donne[31] (cf. Kolmer,[86] Hoffmann,[58] Ingraham[59]) described a regularly spiraled micro-organism in the exudates of primary syphilitic lesions of the genitalia, Schaudinn and Hoffmann[140, 141] recognized a pale, similarly spiraled organism in primary lesions and inguinal lymph nodes of syphilitic patients. Donne, who published his findings in 1837, believed that he had discovered the infective agent of syphilis. His observations were confirmed by Vanoye[156] in 1841 in a report that has apparently escaped the attention of syphilographers. Vanoye found the identical organism described by Donne in lesions of male and female syphilitic patients, and he believed that the identification of these organisms in genital lesions could be utilized as a method for the diagnosis of syphilis. Little credence was initially given to the earlier work of Donne and of Vanoye, but following the rediscovery of the organism by Schaudinn and Hoffmann, investigators in widely scattered centers soon confirmed the presence of spirochetes in early active lesions of syphilis (Russia: Zeleneff[165, 166]; Scotland: Taylor[152]; France: Brunet[18], Bodin[15], Levaditi[75]; England: Dudgeon[32]; Germany: Davidsohn[27], Giemsa[47]; Sweden: Almkvist and Jundell[3]; Argentina: deElizalde and Wernicke[26]).

Demonstration techniques

Numerous methods for demonstrating the organism were rapidly investigated after the spirochete of syphilis was identified by Schaudinn and Hoffmann. These techniques may be divided into two distinct groups. In the first, the spirochete is impregnated with a dye or metallic ion such as silver and rendered visible against a pale background. With the second technique, the background is stained black, or the illumination is altered so the background is darkened, and the unstained spirochete appears pale and sharply outlined against the dark background. The darkfield illumination method is based on this principle.

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The early staining techniques utilized the dyes commonly known as aniline dyes or coal tar derivatives. Generally in the case of the spirochete of syphilis some form of mordant is required to fix the dye, for in the absence of a mordant the spirochete is stained only slightly or not at all. Staining practices were first limited to smears, but subsequently techniques were devised for staining the organisms in histologic sections. Schaudinn and Hoffmann[140, 141] utilized an azure-eosin mixture to demonstrate the spirochete, first in primary lesions, and finally in lymph nodes of syphilitic patients. In the same year Giemsa,[47] using a similar mixture, likewise succeeded in staining spirochetes. Later he suggested minor improvements on his technique.[48] Subsequently Bruckner,[27] Davidsohn,[27] Dudgeon,[32] Ballenger,[8] Tilden,[153] Mühlpfordt,[119, 120] and more recently Olsen and Weller[100] successfully employed a variety of aniline dyes together with mordants.

Various forms of polychrome methylene blue have been utilized by several investigators including Bruckner, and Olsen and Weller. Other aniline dyes studied were the following: azure-eosinates (Schaudinn and Hoffmann, Giemsa, Bruckner), gentian violet (Tilden), basic fuchsin (Tilden, and Olsen and Weller), Victoria blue (Mühlpfordt), and cresyl echt violet (Davidsohn). At one time or another practically all the dyes utilized by the histologist have been employed in efforts to stain the spirochete. In all cases simple aniline dyes alone have not succeeded in staining the organism sharply, and only when a suitable mordant was employed was the stain at all reliable. The mordants were for the most part known protein precipitants such as phenol, tannic acid, acetic acid, phosphotungstic acid, and phosphomolybdic acid. With the resulting chemical alteration of the organism, the dye finally becomes at least partially fixed, revealing a sharply defined, generally more brilliantly stained spirochete against a pale but often stained background. Staining methods using aniline dye derivatives were never as successful as the later forms of metallic impregnation, since they were frequently inconsistent and usually showed considerable fading (Coles[25]). One method described by Olsen and Weller[100] utilized the mordant action of phosphomolybdic acid together with Unna's alkaline methylene blue, and carbol fuchsin or carbol iodine green. This staining procedure is probably the most satisfactory of the techniques utilizing the coal tar dye derivatives, although it also may give inconstant results.

Because of the general lack of consistency of staining techniques employing aniline or coal tar derivative dyes, a search for better staining methods was made. This soon revealed that impregnation with silver salts resulted in specific staining of the spirochete. In 1905, shortly after the discovery by Schaudinn and Hoffmann, Bertarelli, Volpino, and Bovaro,[13, 14] utilizing methods of silver impregnation of nervous tissue, demonstrated the Spirocheta pallida. In the same year Levaditi[75] and Petresco[121] devised similar techniques and likewise demonstrated the organisms. It was found that silver salts were reduced to metallic silver, resulting in sharpness of outline and marked contrast of the black or dark brown spirochete against the yellow hue of the background. The silver impregnation techniques were far from perfect, and the results varied widely. Artifacts, silvering of the sections, and inconsistency due to technical carelessness or impurity of chemical reagents occurred.

Special techniques for staining smears were devised by Fontana[39] and subsequently improved by Fontana,[40] Tribondeau,[154] and Hage.[50] Newer and preferred smear techniques have more recently been developed by Warthin and Starry,[162] Steiner,[145] and Krajian.[67] Silver impregnation techniques when applied to smears have for the most part resulted in atypical forms with marked changes in the regularity and shape of the

spirals, probably due to the drying and flattening of the organisms because they are unsupported by the stromal framework in tissue sections, or by the liquid vehicle in darkfield preparations.

Methods for staining spirochetes in histologic sections were devised for both single sections and block impregnation. Single section techniques have been utilized by many investigators because of the shorter processing time required, but block impregnation techniques have been found to be more reliable and show fewer artifacts (Turner[155]). In single sections as in smears following impregnation with silver, a considerable amount of reduced silver is deposited in and upon the sections, resulting in numerous black artifacts. In some single section methods silver nitrate has been used alone or in loose combination with proteins such as albumin. Bergel[11] believed the staining qualities of the spirochete to be related to changes in the lipoid nature of its cell wall as a result of the action of the strong alcohol frequently used in the processing. Other methods obviously alter the structure of the spirochete during staining, but there does appear to be some relationship between lipoid structure and the staining qualities of the organism. Of the single section techniques utilizing silver impregnation methods, those of Dieterle,[30] Nieto,[121–123] and Jahnel,[61–63] have been found to be the most consistent and satisfactory. The Warthin-Starry[159–161] techniques have been popular although they have shown inconsistency in results in some laboratories. Other methods have been developed by a number of investigators including Steiner,[149] Gyenes and Sternberg,[49] Armuzzi and Strempel,[9, 150] and Krajian.[68, 69] The use of metals other than silver for spirochetal impregnation was investigated by Ghoreyeb,[45, 46] who tried salts of lead and osmium. These methods are apparently not as satisfactory as the ordinary silver techniques.

Silver impregnation of entire blocks of tissue, while time consuming as compared with the twenty-minute staining processes of Krajian, offers greater freedom from artifacts and greater reliability. The methods of impregnation were for the most part devised, utilized, and improved by the French school under Levaditi.[78] Modifications in technique were made by Levaditi and Manouélian,[83] Manouélian,[82] Nyka,[127] and Haythorn.[52]

Staining or blackening the background to allow the unstained spirochete to stand out palely in contrast with the surrounding areas was studied by Burri.[19] Burri's method utilized India ink and has been investigated at one time or another by many students of syphilis. Cohn[23] and Barach[9] were among the early investigators. In addition to India ink, several dyes including Congo red (Benians[10]) and nigrosine (Dienst and Sanderson[29]) were similarly used. A method utilizing collargol was devised by Harrison.[51]

The most common and most widely employed method of demonstration of the Spirocheta pallida involves the alteration in the angle of lighting by special substage condensers. This method, known as the darkfield illumination technique, shows the spirochete in the motile, viable state, while all others reveal the organisms after they have been killed by fixation and manipulation. The darkfield techniques were studied carefully by many investigators, including Antoni,[1] Cares,[21] Coles,[24] and Hoffmann.[53–56]

A more recent but cumbersome method of demonstration of the Spirocheta pallida utilizes the electron microscope (Zworykin *et al.*[197]) which gives magnifications of between 4,000 and 40,000. With this technique many structures not seen by routine microscopy are visualized. Structures noted by early investigators with their staining techniques and long regarded as artifacts, such as flagella and intraspirochetal refractile

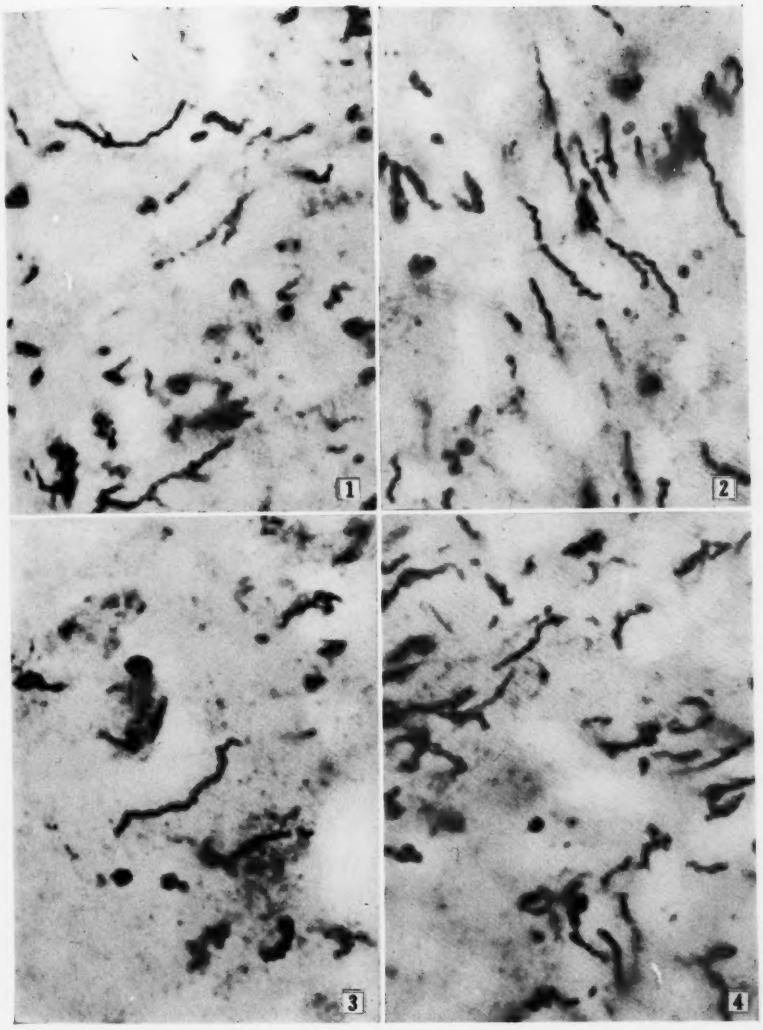
bodies, have been demonstrated through electron microscopy by Wile,[163, 164] Mudd, Polevitzky and Anderson,[117, 118] and Morton and Anderson.[115, 116] Phase contrast microscopy has also been recently employed.[26, 62]

Morphology of the Treponema pallidum

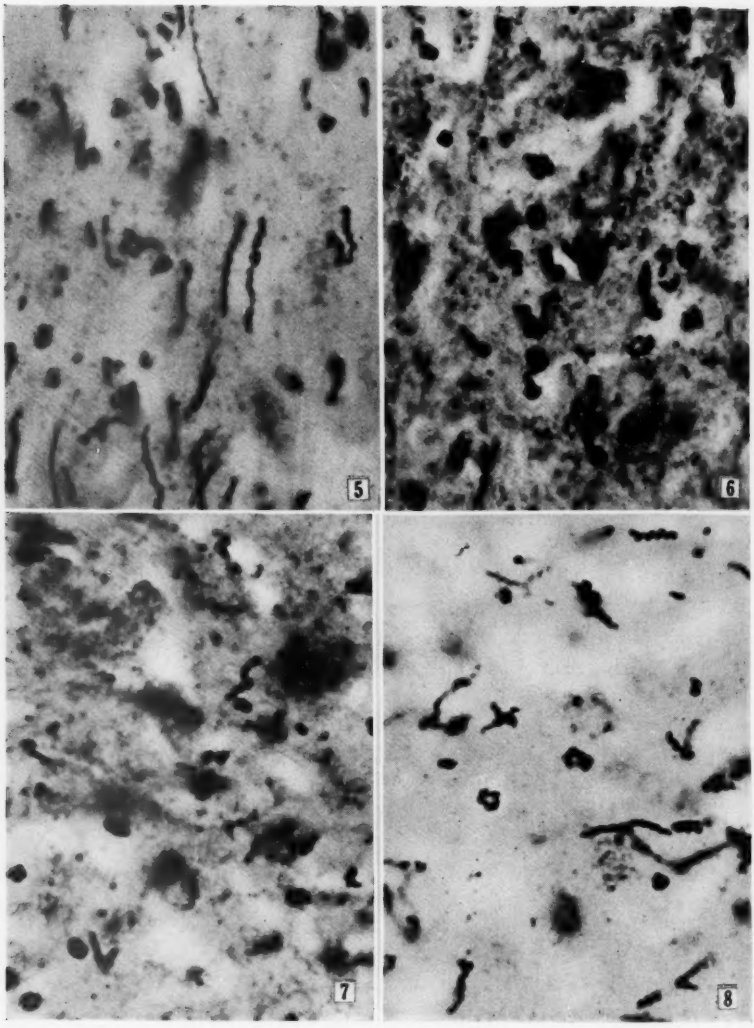
Soon after Schaudinn and Hoffmann described the spirochete, which measured between 6 and 14 microns in length, a number of investigators began to note shorter and longer forms and finally varieties of shapes. Within two years most of the forms ascribed to the evolution or involution of the spirochete of syphilis had been described by Krzystalowicz and Siedlicki,[70, 71] Bosc,[16] Berger,[12] Jacquet and Sézary,[60] Ciuffo,[22] Ewing,[35] Provazek,[132] and Sakurane.[136, 137] Of the forms considered to be part of the cycle of evolution are the so-called buds which are described as small oval argentophile structures attached either terminally or laterally. These buds are occasionally separated from the spirochete by a delicate filament or stalk, and sometimes they are free within the tissues. The investigators who considered them to be developmental forms or buds were Buschke and Fischer,[20] Meirowsky,[105–113] Kermorgant,[64, 65] Leishman,[73] Noguchi,[124, 125] and Geistfeld.[44] Others who demonstrated the forms included Nyka,[127–129] Manouélian,[96–97] Levaditi,[77] and Warthin and Olsen.[157, 158]

A second variant has been known as the granular form. The intracytoplasmic variety has been described in lymphocytes, histiocytes, large and small giant cells, and in fibroblasts. The granules are generally from 0.3 to 0.4 microns in diameter, and are best demonstrated by special modifications of the silver stains including those of Nyka, and Warthin and Olsen. These forms have been described by McDonagh,[98–104] Nyka,[127–129] Warthin and Olsen,[157, 158] Saleeby and Greenbaum,[138] Levaditi, Sanchis-Bayarri, and Schoen,[89] and Ehrmann.[34] The extracellular granular form is generally larger and varies more in size. The granule is described as round or oval measuring from 0.3 to 1.0 microns in diameter and is likewise argentophile. This form has also been described by many investigators including Balfour,[4, 5, 6, 7] Fantham,[36–38] Seguin,[143–145] Hoffmann,[57] Levaditi,[89] Warthin and Olsen,[157, 158] Manouélian,[96–97] McDonagh,[98–104] Noguchi,[124–126] Nyka,[127–129] and Gastinel and Mollinedo.[48] This type is seen routinely in smears and sections stained with any silver impregnation technique. Several early investigators described delicate small filamentous spirochetes arising from the granular forms, i.e., Balfour,[4, 5, 6, 7] Leishman,[73] Fantham,[36–38] and Sergent and Foley.[146] This occurrence has neither been confirmed nor recorded in recent literature.

A third group of forms commonly found in sections includes a variety of ring types which may be small or large, intracellular or extracellular,



FIGS. 1 TO 8: Spirochetal forms observed in an active syphiloma of the rabbit. All photographs taken at 1500 x magnification by Mr. Howard J. Reynolds. A variety of spirochetal forms are shown including the following: filamentous forms, short forms, irregular forms, thick long forms, circular forms, forms with terminal ovoid body, free ovoid bodies, incomplete serrated circular forms, comma forms, intracellular circular smooth and serrated forms, extracellular granular circular forms, and granular forms.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regular or irregular, stellate, rectangular, oval, elongate, serrated, or smooth. These have been described by Fouquet,[41, 42] Seguin,[142–144] Warthin and Olsen,[157, 158] Levaditi, Sanchis-Bayarri and Schoen,[89] Nyka,[127–129] and Sezary.[147]

A fourth group includes thin filamentous and beaded filamentous forms which stain only very palely, if at all, by the usual silver impregnation technique. These occur both intra- and extra-cellularly. They have been best demonstrated by Nyka,[127–129] and have also been noted by Manouélian,[96–97] and Levaditi, Sanchis-Bayarri, and Schoen.[89]

A final group of atypical forms has been hypothecated by Lepine,[73, 74] Levaditi, Sanchis-Bayarri, and Schoen,[89] Warthin and Olsen,[157, 158] and others to explain infectivity in certain conditions in which no visible form of spirochete can be demonstrated. This has been known as the ultramicroscopic or invisible type.

Life cycle theories

There has been much speculation on the significance of the various types of argentophile structures in the tissues of syphilitic patients in association with spirochetes and seemingly having a stream of relationship with them. From the beginning these forms were generally considered to be atypical and involutional. A few investigators, however, believed that the reverse was true, and from their interpretations arose schools of the evolutive cycle, i.e., those of Meirowsky, McDonagh, and of Levaditi. The investigations of these workers and their associates have attempted to fit the various forms into some kind of developmental cycle, but none of the theories has satisfactorily explained the structures or the conditions as they exist.

The simplest hypothesis of the evolutive cycle was proposed by the bacteriologist, Meirowsky.[108, 109] His original theory was based on the presence of a so-called bud form which had been frequently demonstrated in both darkfield and silver stain preparations. The "buds" were small oval argentophile globules attached to the mother adult form either terminally or laterally. Occasionally the "bud" was found attached to the spirochete by a thin filament or stalk, but sometimes it was free in the tissues or examined material. According to this theory, separation of the globule from the spirochete results in a "bud" from which typical adult forms arise. The newly derived spirochetes exhibit the usual delicate regular spirals of eight to twelve undulations, and then proceed by further budding to increase in number. The theory of evolution by budding forms was subsequently restudied and further elaborated on by Meirowsky.[111–118] These buds were also described by other investigators including Antoni,[1] McDonagh,[86–104]

Levaditi, Sanchis-Bayarri, and Schoen,[89] Levaditi,[77, 78, 81, 82, 84–87] Saphier,[139] Szilvási,[151] Warthin and Olsen,[157, 158] and Noguchi,[124–129] but there was no agreement as to their significance. Meirowsky considered the spirochete of syphilis to be one of the higher fungi.

A second more complicated theory of the evolutive cycle was expounded by McDonagh.[96–104] He classed the spirochete with the Protozoa, and his theory of the evolution of the spirochete parallels the development of the malaria parasite. According to McDonagh the life cycle begins with small granular forms or bodies demonstrated within the endothelial cells. These he designates as "sporozoites." The sporozoites or intracellular granules are considered to be the infective agent of syphilis. Development continues with the nuclear budding of the intra-endothelial sporozoites giving rise to a merozoite form. Further development of the merozoite form follows, with the formation of immature male and female forms. The endothelial cell walls subsequently burst, freeing these forms which further mature and by a process of fertilization begin the evolutive cycle once again. McDonagh claimed that he had been able to follow the complete cycle many times although no other investigator has substantiated his claims. Many workers have observed the small intracellular granules, not only in endothelial cells, but also in lymphocytes, fibroblasts, giant cells, and in other macrophages (Ross,[133–135] Moolgavkar,[114] Lundie and Goss,[91] Levaditi *et al.*,[77–86] Warthin and Olsen[157, 158] and Nyka[127–129]), but no investigator has been able to demonstrate the complete cycle.

A third evolutive cycle was proposed by Levaditi[88, 89] in 1927, and subsequently elaborated upon in 1928. This cycle was based entirely upon the morphological variations of the spirochete as demonstrated by many investigators. From the apparent changes in size, shape, and argentophile nature, a coherent scheme was evolved, with recognizable forms, each apparently completing a phase in the cycle. Subsequently, Levaditi turned away from his evolutive theories, but he left intact a sequence which may be ascribed without change to involution. Levaditi divided the spirochete into two phases: The first was composed of typical, regularly spiraled organisms, and the second of all the atypical forms. The atypical forms were considered developmental stages in the evolutive cycle. They were for the most part located intracellularly in the tissues, being found in giant cells, fibroblasts, and macrophages. The groups which he described are as follows: (1) Filamentous flattened forms with slightly bulging extremities; (2) Shortened club or dumb-bell forms; (3) Incomplete loop or ring forms; (4) Complete loop and ring forms; (5) Compact ball forms;

(6) Comma or question mark forms, with or without attached delicate filaments; (7) Marked argentophilic granules, ranging in diameter up to 3-4 microns; (8) Ultramicroscopic granular forms. Most of these variants have been observed by many of the aforementioned investigators.

Lepine[73, 74] hypothecated the existence of a virulent virus or ultramicroscopic organism as the actual cause of syphilis. According to this theory the spirochete is an avirulent organism. However, ultra-filtration experiments made by Lisi[90] and Levaditi[89] utilizing various filters, i.e., Chamberland Types L-1, L-2, and L-3, collodion membranes, and others have been unsuccessful in demonstrating a filterable virulent form.

Experimental

In an attempt to identify and photograph the various atypical forms of spirochetes found in syphilomas of the rabbit, a number of special techniques for silver impregnation and reduction were restudied. Staining single sections by the methods of Warthin and Starry, Nieto, Dieterle, and others, resulted in dark brown to black organisms outlined against a yellow to yellow-brown background of tissue structure. Photomicrographs of these preparations were lacking in sharpness, and besides, the sections often were spotted by a heavy precipitation of metallic silver. Only extreme uninterrupted care with each individual section throughout the techniques produced satisfactory results. The silver precipitation noted with the single-section technique occurred over the surface of the block and did not penetrate to the deeper levels. It therefore could be eliminated by cutting the sections well below the level of precipitation, but photomicrographs of the smaller atypical forms were difficult to obtain because of the colored background, and also because many of the forms were not revealed by these techniques.

The block techniques of Levaditi were generally more successful and required considerably less care during processing. One drawback was the relatively prolonged time required for impregnation and reduction of silver in the tissue blocks. An attempt to reduce the time through the use of alcoholic solutions was made with great success, but with alcoholic silver impregnation there occurred a precipitation of the metal in deeper levels of the tissue. Moreover, it was noted that exposure of the blocks to light during silver impregnation and reduction increased the rate of reduction to metallic silver, with resultant increased numbers of black silver artifacts. The adoption of light-free techniques and the use of amber bottles for the processing solutions effectively diminished the number and distribution of silver artifacts.

The next study was directed at efforts to reduce the intensity of the diffuse staining of the tissue framework and thus increase the sharpness of outline of the organisms. Much of the diffuse tissue staining appeared to result from the action of the reducing agent itself, rather than of the silver salt. Pyrogallic acid which Levaditi employed stains tissues yellow on oxidation, and hydroquinone, another reducing agent, also stains tissues yellow but to a lesser degree. A less soluble and less active reducing agent than these seemed indicated, and therefore p-hydroxyphenyl-glycine (photoglycine) was chosen for study. Alcoholic and aqueous solutions of this reducing agent were employed. It was found that although the solubility of photoglycine was much less in alcohol than in water, more rapid penetration was obtained with the alcoholic solutions. The results were even more satisfactory when sodium sulfite was added to the developer in order to stabilize it.

These changes in the technique of impregnation and reduction resulted in a gray-green diffuse tissue background instead of the usual yellow color of the classical methods. The spirochetes, however, were stained a deep blue-black, and many of the atypical forms were easily identified, indicating that a considerable degree of silver impregnation had been obtained. The gray-green tissue background was a distinct disadvantage. Attempts to clear the background without too great diminution of the spirochetal stain were then made. For this purpose several reducing agents frequently used in photography were tested. Both potassium ferricyanide and potassium permanganate gave good results, but potassium ferricyanide was found to be the best of the chemical reducers tested. Clearing with potassium ferricyanide was therefore adopted as an integral part of the processing technique. The entire section could be cleared of the stain, the spirochetes and atypical forms appearing as densely stained blue-black bodies against a colorless background. With the clearing of the background most of the atypical forms could be photographed with comparative ease.

From these experiments the following procedure was finally adopted:

1. Fix tissue blocks 2-5 mm. in thickness for 12-24 hours in buffered 10% formalin.
2. Transfer and wash tissue blocks in three changes of distilled water for 30 minutes.
3. Transfer to 95% ethyl alcohol for 12-24 hours.
4. Transfer to and incubate at 37° C. in a 3% silver nitrate solution in 50% ethyl alcohol in distilled water for 12-24 hours (amber bottle).
5. Transfer to and wash tissue in three changes of distilled water (amber bottle).

6. Transfer tissue to developer solution containing stabilizer for 12-24 hours at room temperature (0.5 gms.% photoglycine and 0.5 gms.% sodium sulfite dissolved in 50% ethyl alcohol in distilled water). Prepare fresh developing solution for each group of tissue blocks and filter before use.

7. Transfer tissues to and wash for one hour in three changes of distilled water.

8. Transfer tissue blocks to 80% alcohol (2 hours), 95% alcohol (2 hours), 100% alcohol (2 hours), aniline oil (one hour or until tissue blocks become transparent and sink), xylol (three changes, one-half hour, 1 hour, 1 hour), paraffin (two changes, 1 hour, 1 hour), and embed. Retain tissues in amber containers to prevent reduction of silver by light.

9. Block and cut sections at five microns. Mount on albuminized slides, fix by heat in paraffin oven.

10. Remove paraffin in xylols (3 changes) and pass through alcohols (100%, 95%, and 80% ethyl alcohol) to water.

11. Cover sections with freshly prepared solution of 1.5% of reagent grade potassium ferricyanide. The section changes from gray-green to colorless or cloudy white in from one to two minutes. Slight prolongation of time does not change the apparent silver content of the spirochetal forms.

12. Wash sections in three changes of distilled water (1 to 2 hours).

13. Transfer through and dehydrate in ascending alcohol concentrations, clear in xylol, and mount in Clarite or permount.

Spirochetes and spirochetal forms appear blue-black against a colorless background.

Innumerable tissues have been processed by the above technique. From this experience we have observed that several variables can be introduced without altering the end results. Tissue may be fixed for 24 hours or longer in neutral 10% formalin. It may be stained for from 24 to 72 hours in the silver solution, and allowed to remain in the developing solution for from 24 to 72 hours. The background may be decolorized either after the tissue has been cut and dried on slides, or immediately at the time of cutting by floating the ribbon on the warm decolorizing agent before mounting on slides. This latter method is especially desirable when time is an important factor for it saves eight steps in the handling of the slides. Fixation in acetone, alcohol, acid formalin, or alkaline formalin all proved unsatisfactory. Alkaline silver was also unsatisfactory.

The following schedule is used with excellent results: Fresh tissue is placed in the refrigerator at 5° C. for several hours. This prevents shrinkage. The tissue is then fixed in buffered 10% formalin for 24 hours. The blocks are trimmed and washed in distilled water for 30 minutes and then placed in 95% alcohol for 16-20 hours. A fresh solution of 3% silver

nitrate in 50% alcohol is made in a clean amber bottle, and the blocks of tissue are dropped into this where they remain at 37° for 24-48 hours. The solution is poured off and without removing the blocks from the bottle, they are washed in 6-8 changes of distilled water for 1 hour. This is done in the dark. The developer solution is made by dissolving 0.5 gm. of anhydrous sodium sulfite in 50 cc. distilled water. When this is dissolved, 0.5 gm. of photoglycine (p-hydroxyphenyl-glycine) is added. The bottle is shaken for several minutes until all of the photoglycine which will dissolve has gone into solution. Then 50 cc. of 95% alcohol are added, a few cc. at a time and with constant shaking. The solution is then filtered directly over the blocks of tissue which have been well drained of water. The bottle is kept at room temperature, in a dark closet for 24-48 hours. The solution is then poured off and the blocks are washed in three changes of distilled water for one hour. The tissue is then dehydrated, cleared in xylol, embedded in paraffin, and cut at 5 microns.

For decolorizing before mounting on slides, the ribbons of tissue are separated into suitable lengths for mounting, and transferred to 1.5% aqueous solution of potassium ferricyanide which has been warmed to about 54° C. When the sections appear opaque, they are transferred to two changes of distilled water to remove the decolorizing agent. They are then mounted from warm water on albuminized slides. The slides are allowed to dry in the paraffin oven, the paraffin is removed with xylol, and coverglasses are applied over Clarite. For decolorizing after mounting on slides and drying in the usual manner, the paraffin is removed and the slides carried through alcohol into distilled water before being placed for one or two minutes in the potassium ferricyanide solution. When the background is clear or opaque, the slides are washed in several changes of distilled water, dehydrated, cleared, and mounted. There is no appreciable difference in the end results with either method of decolorization.

If desired, counterstaining with hematoxylin and eosin can be performed as a final step. This does not decrease the intensity of the spirochetal stain and permits histologic study of the lesion on the same tissue block used to determine the presence of organisms.

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CERTAIN ASPECTS OF ACUTE INFECTION OF THE RESPIRATORY TRACT

A. RAYMOND DOCHEZ

Many years have passed since Flugge first drew attention to the probable importance, in the transmission of infection by way of the respiratory tract, of material expelled into air by infected individuals during the acts of talking, coughing, or sneezing. Infective particles disseminated in this manner manifest themselves in two forms: the moist state, in which many of the particles are relatively heavy and settle rather rapidly a short distance from the point of origin; and the very small particles which dry quickly and remain suspended in the air for variable periods of time, the so-called droplet nuclei. The former may rise again into the air in the form of dust resulting from the disturbance, by one means or another, of the surfaces upon which they rest. Viability and infectivity of the microbiological agents contained within the dried material are of variable duration, depending upon a number of environmental conditions. Distribution is easily promoted by air currents and the infectious material may travel considerable distances.

Another probable and important mechanism of transmission of infection of the respiratory tract is by direct contamination of articles of common use, the handling or use of which may permit infectious material to establish contact with the upper respiratory tract. A striking example of this is the heavy contamination by infective agents of such articles as blankets, which, on being moved or shaken, disperse in the living state large numbers of the organisms with which they are contaminated.

The chief portal of entry of infectious microorganisms into the human body has at all times been the respiratory tract. This is readily understandable if we bear in mind that this physiological system is continuously open to all floating matter in the air. That it is partially protected by sticky, mucous membranes lining tortuous passages, by aggregation of lymphatic tissue; by ciliary movement along tracheal and bronchial mucosae is true; but one has only to examine the lungs and regional lymph nodes to discover how much foreign material penetrates the protective barriers and reaches the deeper tissues.

Unfortunately, infection of the respiratory apparatus is spread, for the most part, directly from individual to individual by the necessary and continuous association of human life. At present there is no discernible

tendency of acute infection of the respiratory tract to diminish in incidence or in the extent of its geographical distribution. If science could bring this type of infection within the sphere of effective public health control, great benefit would accrue to humanity. In attempting to solve a problem of so complex a nature it is necessary to study carefully, patiently, and with a determination not checked by years of apparently fruitless effort the character and succession of events which are manifest in outbreaks of the diseases under discussion. Only by such intensive study will the points of vulnerability of this most strongly intrenched system of diseases be brought into the light of human understanding, and perhaps within the scope of successful attack by the forces of preventive medicine.

Infection of the respiratory tract (leaving out of account uncommon types) is, in general, due to two different classes of microorganisms, the filterable viruses and the bacteria. Three important varieties of respiratory infection are known to be caused by filterable viruses—the common cold, of which there are probably several kinds, influenza, and primary atypical pneumonia, commonly called virus pneumonia. Of the bacteria which cause infection of the respiratory tract the most important are pneumococcus, hemolytic streptococcus, and, less frequently, Haemophilus influenzae and Staphylococcus aureus. I shall leave out of account such specific diseases as measles, whooping cough, and diphtheria and the secondary organisms which frequently complicate these affections.

Of the filterable viruses, those of the common cold and of influenza cause primary infection of the upper respiratory tract and that of primary atypical pneumonia infection of the lungs, though the latter virus may cause infection of the upper respiratory tract without pulmonary involvement. Bacterial disease of the respiratory tract manifests itself principally as infection of the pharynx, tonsils, paranasal sinuses, and lungs. A considerable variety of bacterial species serves as the causative agents of these latter infectious processes. Many of these bacterial infections of the respiratory tract appear to be primary in character, and without doubt a considerable number of them are. Taken altogether the agents of respiratory infection are very numerous and are variable in respect to their biological characteristics and the location of the disease which they produce.

At this point one may ask whether respiratory disease consists of a hodge-podge of events, indicating a series of accidental encounters of the individual with pathogenic microorganisms, or whether there has evolved within this complex system of diseases a pattern more or less consistent and representing a series of interdependent relationships each of which is

important in sustaining the present impregnable position of acute infectious disease of the respiratory tract. If the latter assumption should prove in many instances to be a true one, then analysis of the pattern and persevering study of its individual parts may well lead to knowledge that would be useful in influencing favorably the incidence of respiratory infection as a whole. Many reliable observations have been made which indicate interdependent bases of activity on the part of certain orders of microorganisms which cause infection of the respiratory tract.

A helpful generalization in understanding the sequence of events in respiratory infection is a division of the causative agents into those of a primary and those of a secondary order, bearing in mind, of course, that each of these orders may be capable of inducing primary disease of the respiratory tract unassisted by the other. As a rule, however, it would seem more commonly to be the case that, in order to produce the complex picture of respiratory infection, a certain interdependent activity between the two groups of organisms is necessary. Members of the primary order would seem to be the etiological agents of the common cold, influenza, and allied conditions which initiate the pathological process, and members of the secondary order, the common pathogens of the respiratory tract which intensify and extend the harmful activity. For a thorough understanding of the problem of acute respiratory disease the relationship, both in time and character, of activity of all the agents concerned is essential. The most important of the primary factors are the causative agents of the common cold and influenza. I shall leave out of account the virus cause of primary atypical pneumonia, since this virus produces a primary pneumonitis generally unassociated with secondary bacterial infection, and the total extent and variety of its activity has not as yet been determined. The most important field of investigation at the moment seems to me to be the nature and mode of action of those viruses which I have designated as the primary agents of acute respiratory infection.

In a sense the common cold may be considered the keystone of the arch composing the types of acute respiratory infection under consideration. Its primary activity and secondary effects are fairly representative of the total picture of acute respiratory disease. Before taking up the specific etiology of the common cold a short consideration of some of its epidemiological aspects is of importance as a background. As everyone knows, this malady is the disease from which human beings most frequently suffer. It prevails more or less extensively in all climates, and susceptibility is practically continuous from infancy to old age. A single attack seems to leave little or

no immunity against subsequent infection, and its subjects may suffer from one to six or seven attacks each year. The common cold may be considered in its simplest form as a rather mild disease of the upper respiratory tract of from three to five days' duration. This simple form of the infection seems, however, to be a comparatively rare occurrence since there nearly always occurs more or less secondary activity on the part of organisms not responsible for initiation of symptoms. The latter activity manifests itself as sinusitis, pharyngitis, tracheitis, or bronchitis; or occasionally as inflammation of the middle ear, or pneumonitis of varying severity. The bacteria participating in these inflammatory processes are the common potential pathogens, the pneumococcus, streptococcus, staphylococcus, and H. influenzae that at some time during the winter months are usually present in the upper respiratory tracts even of healthy individuals. Associated activity of this kind is a notable feature of certain animal diseases, such as canine distemper and swine influenza, in which the relationship of the primary viral agent to the secondary bacterial agent has been definitely established.

Such a direct relationship is not so clear-cut in infection of the human respiratory tract. Nevertheless, there is justification for assuming the existence of a similar type of associated activity in human infection. The occurrence of various kinds of suppurative inflammation of the upper respiratory tract and of lobar and bronchopneumonia as sequels of the common cold offers convincing evidence of the validity of this assumption.

Consideration of the epidemiology and bacteriology of the common cold in representative population groups shows well-defined peaks in the curve of morbidity. These peaks are typically, but not always, three in number and occur in the early fall, mid-winter, and mid-spring months. The severity of the disease tends to vary in the different outbreaks, the mid-winter one usually exihibiting the greatest severity and the largest number of complicating infections. For example, the number of infections sufficiently severe to cause absenteeism from work is usually much larger during the January outbreak of colds than during the October outbreak.

A study by Kneeland and Dawes* throws an interesting light on these phenomena. These investigators kept the infant population of a foundling home under continuous observation for a number of years in order to study the character of respiratory infection in such a group and its relationship to the distribution of the common pathogenic organisms of the upper respiratory tract. For this purpose frequent cultures were made from the

* Kneeland, Yale, Jr. and Dawes, Caroline F.: J. Exp. M., 1932, *55*, 735.

nose and throat and, in addition, changes in skin sensitivity to prevailing pathogens were noted throughout the period of study. A record was also kept of the number and character of all acute infections of the respiratory tract.

There occurred the usual cyclic outbreaks of acute upper respiratory infection. In the early fall the percentage of individuals giving significantly positive skin reactions to the various bacterial products used was relatively low, approximately 15 per cent. As the winter progressed, the positive skin reactions became more frequent and the intensity of the reactions increased until in the late spring 85 per cent of the group under study gave significantly positive skin reactions. This increase in number of positive skin reactions was interpreted as indicating increasingly frequent and widespread infection with the common pathogens of the respiratory tract. As a rule, the individuals suffering from the greatest number of infections and those harboring the organisms in their respiratory tracts for the longest period of time were found to exhibit the most strongly positive skin reactions.

The incidence, distribution, and type of pathogenic organism found by culture varied from time to time. During the preceding year the carrier rate of pneumococcus rose for a short period of time to the exceedingly high figure of 95 per cent. Throughout the periods of widespread distribution of pathogenic organisms, the character of much of the respiratory disease observed was severe and of the type of grippe, influenza, and pneumonia. On the other hand, when the distribution of pathogenic organisms was relatively limited the character of the respiratory infection was mild and of the type of the common cold. A similar situation is believed to account for the mildness of the common cold in the tropics.

These observations have been presented in some detail since they seem to represent fairly accurately the annual progress of events in acute infection of the respiratory tract. From September to May, the period of high incidence of upper respiratory infection, there occur frequent cycles of the common cold and related affections. As a result of these outbreaks increasing opportunity is afforded the pathogenic organisms of the respiratory tract to cause secondary infection. As a sequence of this, distribution among the population at large is facilitated and virulence perhaps enhanced, a phenomenon that becomes more and more evident as the year progresses through the cold winter months into early spring. Though this may be but a part of the total occurrences involved, the conclusion seems justifiable that epidemics associated with the severe forms of respiratory infection may

result from the coincidence of an outbreak of common cold or influenza with a period of widespread distribution and increased virulence of the well-recognized pathogenic organisms of the respiratory tract. Such efforts are well illustrated by the pandemic of influenza in 1918 and by the association of measles in military establishments with widespread hemolytic streptococcus pneumonia in 1917-18.

For the development of a successful attack upon this complex disease system from the standpoints of preventive medicine and therapy, several approaches to the problem are possible. In addition to the facts already known much new knowledge is required. Pasteur has implied that given the knowledge of the causative agent of an infectious disease and its means of communication, control of the disease should be possible. This belief has proved valid in the prevention of the previously common infections of the gastro-intestinal tract by means of the sanitary control of water and food, the usual vehicles of these infectious agents. The dictum when applied to respiratory infection presents a problem of far greater difficulty of solution. The most hopeful point of initial attack would seem to be against the viruses of common cold and influenza, which I have presented as the most important primary agents of respiratory infection. The possibility exists that if these two infections could be effectively prevented, all acute respiratory infections might simultaneously diminish as a consequence of the interdependent relationship of the two principal groups of respiratory pathogens, which has been suggested in the previous discussion.

The most direct approach to the control of the viral infections of the respiratory tract would seem to be an attempt to break the line of communication through the medium of the air. Could an effective method of air sterilization be developed, success along this line might be attained. The difficulties of developing a practical and universally applicable technique are great and under certain types of environmental situations almost insuperable. By the use of bactericidal substances, such as triethylene glycol in the form of aerosols, air sterilization can be effected in limited enclosed areas. When applied, however, to large areas with varying environmental conditions and irregular space relationships, effectiveness diminishes to the point of disappearance. At present only limited results can be expected.

Another method of air sterilization is by the use of ultraviolet irradiation. Again this procedure, though effective, has a limited application. Its principal usefulness at present seems to be the prevention of the spread of infection through the air in schoolrooms and hospital wards. Whether either of these two methods can be developed for such large scale use as to diminish

significantly the incidence of upper respiratory infection remains for future investigation to decide.

The goal of all students of infectious disease has been, ever since the time of Pasteur, to control the disease under study by means of prophylactic immunization. Conspicuous success has attended such an attempt in a number of infectious conditions. The method presents an ideal approach to the problem of controlling acute respiratory infection if only the hopefully anticipated results could be attained, namely, immunization of the population against the primary viruses of the respiratory tract with diminution in the incidence of their infectious processes and, as a consequence, a tendency of the secondary bacterial infections to disappear. This approach to the control of acute respiratory infection is being actively explored at the present time, and in certain areas, considerable progress has been made in laying the foundations for the development of practical methods.

The etiology of the most prevalent types of common cold, previously a subject of doubtful and varying explanation, has been determined. By means of experimental inoculation of a certain primate, the chimpanzee, and of human volunteers, typical colds have been produced with the filtered bacteria-free throat washings of human subjects suffering from acute colds, thus indicating the presence of a filterable virus as the causative agent. Some information concerning the nature of this virus has been obtained. The virus has been cultivated in chick embryo tissue culture and in the embryonated hen's egg, so that the first steps toward the preparation of a vaccine have been taken. At this point great difficulties in the way of further advance have made their appearance. Not all investigators have been able to cultivate the virus outside the body and further confirmation of this procedure is important. Determination by *in vitro* or other methods of the presence of the virus in cultures has proved difficult and in the hands of most investigators impossible. No small and easily available laboratory animal has been found to be susceptible to infection with the virus. As a consequence the essential studies concerning the character of the virus, the variety of strains as determined by immunological techniques, and its biological behavior in respect to range of infectivity and capacity for experimental immunization cannot be carried out. Successful re-inoculation of human beings, using the same strain of virus employed for the original inoculation, has been possible after the lapse of three weeks, indicating that little or no immunity results from the primary infection. The natural history of the common cold suggests that a similar situation exists in respect to the spontaneous cold, because of the frequent reinfections that occur at rela-

tively short intervals. The many difficulties that impede the development of an effective method of prophylactic immunization against the common cold make this procedure as a method of prevention a dim and uncertain prospect.

Study of the virus of influenza has encountered far fewer difficulties and much valuable and substantial progress has been made. Cultivation of the virus in tissue medium and the embryonated hen's egg has been accomplished by simple and easy techniques. A number of small laboratory animals can be successfully infected both with material obtained directly from human examples of the disease and from tissue and chick embryo culture. As a result the necessary immunological studies in animals and reactions *in vitro* can be successfully made. By means of these techniques the action of serum antibodies against influenza virus, the development of human immunity to the virus, and the epidemiological significance and importance of different virus strains can all be investigated and dependable results obtained.

Influenza virus in large amounts can be obtained by cultivation in the embryonated hen's egg, and a usable vaccine which, on inoculation, gives rise to demonstrable immunity in human beings has been prepared. Field experiments on a large scale using such a vaccine for purposes of human immunization have already been carried out with some measure of success. Certain difficulties, however, have arisen which make the general use of this vaccine as an effective prophylactic procedure against epidemics of human influenza a matter of some doubt. From the early studies of influenza virus there appeared to be concerned in the production of the disease two well-defined immunological types called Influenza A and Influenza B, one or the other being the predominant strain in successive outbreaks. From this observation the assumption was made that adequate protection would result from the incorporation of the two dominant strains in the vaccine. More recent studies have indicated a wider degree of immunological variation among strains of influenza virus, so that more extensive study of the number of variant strains and of the possibility of natural variation within an established type must be carried out before the necessary components of a potentially effective vaccine can be determined. Whether use of such a vaccine will limit the outbreaks of epidemic influenza, only future trials can decide. The apparently short duration of immunity derived from vaccination or from a natural attack of the disease also raises a question as to the possibility of successful prophylactic immunization against influenza.

In view of the difficulties surrounding the prevention of acute upper

respiratory infection by the classical methods of public health sanitation and prophylactic immunization, should emphasis in the study of these diseases be turned in the direction of therapy? Fortunately, those severe bacterial infections of the respiratory tract, which have been presented as largely secondary manifestations resulting from the effects of an initiating viral infection, can now be generally and effectively treated with a variety of chemotherapeutic and antibiotic drugs. These agents have greatly lessened the seriousness and fatal effects of infection of the respiratory tract with those dangerous pathogens which were such prevalent causes of disability and death in the past. Certain antibiotic substances have already been discovered which exert a curative effect on rickettsial and some virus diseases, including in the latter class primary atypical pneumonia. Studies directed toward this field of therapeutic activity should without doubt be vigorously pursued in the hope that viruses of the type of common cold and influenza may be brought within the range of chemotherapeutic action.

THE SIGNIFICANCE OF THE NON-NEOPLASTIC LESIONS INDUCED IN THE CENTRAL NERVOUS SYSTEM OF DUCKLINGS BY THE VIRUS OF A DUCK VARIANT OF THE ROUS SARCOMA*

F. DURAN-REYNALS

From studies from this and other laboratories it appears that a number of ordinary filterable viruses—and some other infectious agents as well—are endowed with the property of destroying, under certain circumstances, the cells that they infect, and of stimulating them to multiply under other circumstances. One of the factors that determines whether the virus infection will manifest itself as either cell destructive or cell stimulating, that is, as either non-neoplastic or neoplastic, is the existence of a certain degree of resistance linked with aging of the host.

As discussed elsewhere[3, 7] we believe that this concept carries important implications for the cancer problem because it is in the field of avian cancer where the duality of effects of the causative viruses as conditioned by the age of the host is best shown. Thus, when viruses of a variety of chicken sarcomas[4, 8, 15] or of their variants in pheasants, guinea fowls, turkeys, and ducks[5, 6, 9] infect embryos or immature hosts, they induce not tumors but a pathognomonic condition, the hemorrhagic disease, characterized by the development of lesions in the small vessels of a variety of tissues, mostly in viscera. These lesions are the result of the action of the virus on the cells of the vessel wall; the tissue becomes weakened by infection, and blebs form due to pressure within the vessel. These blebs may rupture and lead to death by hemorrhage.

The tissue changes disclosed in these lesions by microscopic study are extremely discrete. Thus, in the hemorrhagic disease of young birds, all that can be seen is the ruptured vessel and the resulting hemorrhage. Occasionally, swelling of the endothelial cells is observed in the earliest recognizable hemorrhagic lesions. In older hosts, proliferation of cells of the liver and spleen sinusoids may be present, frequently independent from hemorrhage; this initial stimulation leads later to the formation of frankly sarcomatous tissue. In the embryo, desquamation and slight swelling of the vascular endothelium is noticed in the hemorrhagic areas while edema and cell necrosis may be present in adjacent tissues. Sarcoma cells were absent in the embryo in all the phases of its development studied.

These non-neoplastic lesions are constantly reproduced by passages of the "tumor" virus through immature hosts. Yet, if extracts of the tissues showing the lesions are injected into older birds, typical tumors develop.

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Other factors besides the age of the host determine the development of either neoplastic or non-neoplastic lesions. One of them is concerned with the quantity or quality of the virus so that larger amounts of virus or a given sample of a more active virus will induce necrotizing preferably to neoplastic lesions. Still another factor is concerned with inherent properties of the infected tissue which will react differently to the virus. Thus, the chorio-allantoic membrane may develop tumors while the embryo does not. Also, the distribution in the chick or the duckling of either tumors or hemorrhagic lesions is characteristically different for each organ. As best illustrating the point, one can name the lung and spleen as the organs most frequently developing tumors and hemorrhagic lesions respectively.

Although tumors of the central nervous system are known to occur in chickens,[2, 10, 12] and although it is known that these hosts react to the intracranial inoculation of the Rous sarcoma virus by developing a variety of sarcomas,[16] neither tumor nor hemorrhagic lesions have ever been detected in the central nervous system in birds of any age, following intravenous injection of tumor viruses or as a generalization from tumors growing elsewhere. This holds true for all the naturally occurring chicken sarcomas and for the vast majority of variants of one of them, the Rous sarcoma, in several species of birds.

However, one of the duck variants of the Rous sarcoma studied by us, sarcoma 14(d)7, was an exception to the rule and stands as a unique case in transplantable avian sarcomas. For the causative virus of this tumor is endowed with the power of localizing itself in the central nervous system of young ducks either after intravenous injection or as a generalization from a primary tumor. On removal of the skull, this localization is strikingly manifested by the presence of large hemorrhagic lesions in the nervous tissues and the meninges.

As adding further to the interest of the phenomenon it developed that, microscopically, these lesions are rather complex, thus revealing events, in the course of the non-neoplastic infection, which are either absent or not noticeable when this particular virus or other tumor viruses infect tissues other than those of the central nervous system.

Materials and methods

The tumor. Variant 14(d)7 of the Rous sarcoma was obtained by injecting newborn ducklings with cell suspensions from a Rous sarcoma grown in a chicken 14 months old. Signs of adaptation to the new species were manifest from the first passage by changes in the gross and microscopic morphology of the tumor, and by the induction of generalized lesions. On further passages the virulence of the virus increased considerably, and the tumor proved to be the most malignant of all the avian sarcomas studied in this laboratory. Both filtrates and cell suspensions injected under the skin or in the muscles of ducklings induced rapidly growing tumors often followed by generalized lesions in viscera and bones and also in the central nervous system. Filtrates injected intravenously were equally effective. However, despite this malignancy, no growth or growth followed by regression was observed when ducks several months old were injected. On the other hand, despite its adaptation to a foreign species, and contrary to what was observed with other duck variants of the Rous sarcoma, the virus of variant 14(d)7 kept, to a large extent, the power of infecting chicks and chickens. In these hosts large tumors were induced, often followed by

generalized lesions much as it happens in ducklings. However, the central nervous system was never found affected in chicks.[9]

Tumor preparations and animals inoculated. Cell suspensions of the tumor consisted of one part of tumor tissue suspended in 4 parts of saline. The filtrates were obtained by extracting 1 part of ground tumor tissue with 19 parts of saline, centrifuging the extract, and filtering the supernatant fluid through a Berkefeld N candle. The cell suspensions were injected in the breast at the dose of 1 or 2 cc. and the filtrates in the vein, at the amount of 1 cc., or intracranially at amounts of from 0.1 cc. to 4 cc.

The ducks and chickens employed were of the Pekin and Plymouth Rock breeds respectively.

The lesions in the central nervous system of ducks. The ducklings showing such lesions were injected with tumor materials at the age of from 1 to 7 days, and they died from 9 to 22 days thereafter. The lesions were first observed at the 3rd tumor passage and then frequently, in the following passages, in ducks that died at or before the age of 23 days; never in birds that died at an older age. Without exception, all the ducks with lesions in the central nervous system also showed a widespread hemorrhagic and neoplastic disease in viscera and other locations. No symptoms attributable to the involvement of the nervous tissue were detected in the diseased ducks.

The lesions in the central nervous system were in the gross always hemorrhagic. They consisted of blebs, some barely visible to the naked eye, some others measuring several millimeters in diameters, and often neatly seen as sprouting from the vessels which appeared distended or thrombosed. In only one case a large blood clot was present underneath the brain. The lesions were observed in all intracranial locations but not in the spinal cord, although the latter organ was not systematically examined (Figs. 1 and 2).

This part of our study is based on material from 33 ducks. In 19 of these the lesions were the result of generalization from primary breast tumors induced by cell suspensions; in the remaining 14 the lesions developed after intravenous injection of tumor filtrates. Sections were taken from portions of tissue in all cases, but in 6 of the ducks serial sections were taken from half of the brain and other intracranial structures. Thus, more than 1500 sections, stained by the hematoxylin-eosin method, were examined, plus another 200 from normal ducklings for comparison.

Microscopically, both in the nervous matter and the meninges, hemorrhage was most conspicuously and frequently observed. It ranged in severity from lesions consisting of a few red cells around a minute vessel to large masses of blood which compressed the surrounding nervous tissue. Frequently no changes were observed in the vessel wall (Fig. 3) except in the site where it ruptured.

In other cases, however, swelling and proliferation of the endothelium, which could lead to partial or total occlusion of the lumen, were observed especially in small vessels, while in the larger vessels adventitial proliferation was more common (Figs. 4 and 5). Images were present suggesting that the vessel walls, whether or not they show proliferation, can undergo necrosis. In the larger vessels this necrosis was not of a massive type but consisted of cells in different stages of disaggregation imbedded in a hyaline matrix especially abundant in the middle vessel layers. In smaller vessels the whole wall showed hyaline degeneration with only a few pyknotic nuclei being recognizable here and there (Figs. 6, 7, 8).

Probably representing a further stage in the evolution of the proliferative lesions just described, clusters of cells were observed as evolving from the endothelium (Fig. 9) and appearing occasionally as emboli (Fig. 10). Generally these cell masses remained within the boundaries of the vessel; only on one occasion were they observed outside the vessel wall. Close examination of the cells composing these clusters revealed, in a few cases, a striking resemblance to the cells from the 14(d)7 sarcomas growing in viscera or developing in the meningeal vessels after intracranial injection of virus. However, these growths in the central nervous system never became any larger than as described so that a diagnosis of sarcoma could never be established with certainty.

Of all the lesions studied those in which only hemorrhage was observed were the most common, and those showing the sarcomatous-like foci of proliferation were the most rare. No relation could be established between the age of the ducks at death and the occurrence of one type or another of lesions. However, it can be affirmed that the ducks injected at the age of one day never showed the sarcomatous-like lesions.

No special effort was made to study alterations in the nervous tissue proper, our interest being mainly focused on the vascular lesions. However, it was quite plain that lesions in the former tissue were present either in the form of diffuse or circumscribed necrosis or in the form of neuroglial stimulation. Whether these lesions were the result of a direct virus effect or secondary to the hemorrhagic lesions one cannot tell.

In 8 cases where the ducks showed lesions in the central nervous system, extracts at 1:20 of this tissue were obtained and each was injected at the amount of 1 cc. into the breast of two ducklings. Tumors, which often became generalized in their distribution, developed in the birds injected with material from 7 of the cases. The extract from the brain in the 8th case proved to be inactive. This particular duck showed minimal lesions.

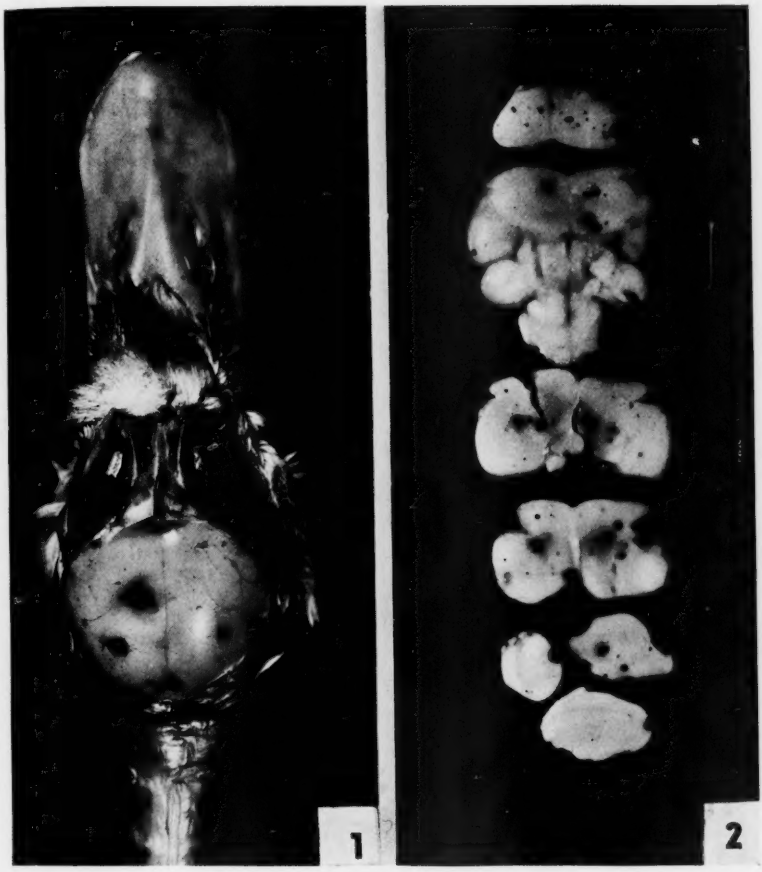

FIG. 1. Gross aspect of the lesions induced by duck strain 14(d)7 after removal of the skull. Mag. x½

FIG. 2. Serial sections of the central nervous system to show the distribution of the lesions. Mag. x½

The tests were repeated employing the nervous tissue of 3 ducklings which showed generalized gross lesions but none in the central nervous system, although no microscopic examination was carried out. The results were that one of the extracts induced tumors, but the other two extracts were inactive.

The lesions in other tissues of ducks. Complementing these observations we studied in serial sections the hemorrhagic blebs which, together with gross tumors, were present in the viscera of some of the ducklings which also showed lesions in the central nervous system. Here, either hemorrhagic lesions, these lesions combined with typical sarcomas, or the latter alone were observed, but endothelial and adventitial proliferation, such as found in the vessels of the central nervous system, was either absent or was negligible in degree, as if the cells reacted to the virus in only the two extreme forms of either destruction or frank malignancy. Nor were the lesions of general thickening of the vessels (followed or not by necrosis) observed in the viscera. Also, the sarcomas in these visceral vessels were observed rapidly invading the neighboring tissues and not limited within the vessel boundaries as in the nervous tissue. In fact, in many of the larger tumors it was no longer possible to trace their origin to cells in the vessels.

The lesions in chicks injected with the duck variant 14(d)7. As previously stated, sarcoma 14(d)7, despite its adaptation to ducks, retains its power to infect chickens. In view of the lesions observed in the central nervous system of ducklings, many chicks, that had been inoculated with the tumor materials either in the breast or intravenously and showed widespread lesions, were studied with the view of ascertaining whether gross lesions, hemorrhagic or neoplastic, were also to be found in their central nervous system. In no case were such lesions found. Microscopic study was carried out only in a few cases, again with negative results.

The intracerebral injection of ducks with tumor viruses. These tests were obviously indispensable for a better understanding of the facts so far described. Eight experiments in all were carried out with the variant 14(d)7 in ducks. In 7 of them amounts of filtrate ranging from 0.1 cc. to 0.4 cc. were injected intracerebrally into a total of 18 ducks from 1 to 25 days of age, the dose of filtrate being in relation to the age of the duck. Twelve of the birds died from 8 to 27 days while the rest were killed later. Tumors in the skull or the scalp developed in 6 of the birds, but in no case were growths observed in the intracranial structures except, in one case, a minute sarcoma in the meninges. However, in all cases congestion of the vessels was present, occasionally accompanied by hemorrhage and endo-

thelial proliferation, but the latter lesions were minimal and far less typical than when the virus reached the central nervous system from the blood. Generalization in viscera or other tissues was never observed. The brains of 2 of the ducks injected intracerebrally were tested for the presence of virus. The birds had died 18 and 25 days before and one of them had a tumor in the skull. Extracts at 1:20 of each of the brains were injected at the amount of 0.5 cc. into 6 ducks. No tumors ever developed.

However, results were different in the 8th experiment. Here 13 ducks, aged from 1 to 4 weeks, were injected with filtrate at doses of 2 cc. for the birds 1 and 2 weeks old, and of 3 and 4 cc. for ducks 3 and 4 weeks old. Surprisingly enough the birds tolerated without obvious immediate ill effects such large amounts of inoculum. The birds died from 13 to 19 days after the injection. In 4 of them clear signs of tumor growth were detected in the meninges, in 1 of them in the form of a rim of tissue grossly visible; in the others as growths around vessels only detectable microscopically. These growths were always accompanied by hemorrhage and, in 2 cases, were strongly infiltrated by myeloid cells. In one case the tumor growth was seen following the course of vessels in a brain fissure, and from these vessels possibly infiltrating the nervous tissue. Also, thickening of the walls of many vessels such as described in the foregoing section was present in this duck. In another 4 ducks there were hemorrhage and thickening of the meninges without detectable tumor growth.

In another 5 ducks ordinary sarcomas developed in the skull, without invading the intracranial structures. In one of them a meningeal growth was present, but without any visible connection with the skull tumor.

Generalization in viscera was observed in 8 of the ducks that showed lesions in the skull or meninges. They consisted of sarcomas or hemorrhagic lesions without any special feature worth mentioning.

In still another experiment each of 12 ducks 1 day old were injected intracerebrally with filtrate from Rous chicken sarcoma at amounts of 0.1 and 0.2 cc. No lesions whatsoever developed.

The intracerebral injection of chicks with the Rous sarcoma virus. In parallel experiments to those just described a first group of 7 chicks, 2 and 7 days old, were injected intracerebrally with 0.1 cc. of a filtrate of the Rous sarcoma virus. Five of the six birds that responded to the inoculation died in from 16 to 25 days thereafter without any gross signs of involvement of either the central nervous system or the skull and skin. Yet, every one of these birds showed in viscera and other tissues the generalized hemorrhagic and neoplastic lesions such as are commonly found following a primary

tumor. In fact, microscopic examination of the nervous tissues showed that such primary tumors did exist in the form of sarcomatous growths developed from the blood vessels. The sixth chick showed in the skull a tumor 0.5 cm. in diameter which frankly invaded the brain.

Extracts at 1:10 of the brain of 3 of the chicks were obtained and each of them was injected either intracerebrally in the amount of 0.1 cc., into 4 chicks, or intramuscularly into the breast of another 4 chicks in the amount of 0.5 cc. From the brains of the chicks injected intracerebrally that died, another group of chicks was inoculated as above and the passages from brain to brain were thus continued, testing at each passage the brain material by intramuscular injection. Several tumor lines were thus obtained, one of them being carried through 5 passages. Results were always the same: in most cases development, in the chicks injected intracerebrally, of microscopic sarcomatous growths in the brain followed by generalization; in a few cases, development in the skull of gross tumors invading the nervous tissue.

Microscopically, in their earliest stages the sarcomatous growths were often observed in association with blood vessels, notably their outer layers. Progression of the growths was following the easiest path along the adventitia of vessels in the sulci and fissures, much as described by Vázquez-López in older chickens.[18] Sometimes, though, masses of tumor cells were observed surrounded by nervous tissue indicating a direct invasion of the latter tissue (Figs. 11 and 12). No lymphocytic reaction was observed. Often there was thickening of vessels, mostly as a result of adventitial proliferation, while lesions of the endothelium generally consisted of desquamation and swelling. The staining methods followed preclude any statement as to whether alterations of the neuroglia and microglia, such as described by Vázquez-López,[19] were present or not.

The Rous virus did not seem to change by the brain passages since the injection of extracts of the brain sarcomas into the breast of chicks resulted in the development of the disease usually induced by the ordinary sarcoma virus.

Discussion

The first point of interest in the present investigation is that a tumor virus, that of the Rous sarcoma, when circulating in the blood, never induces lesions in the central nervous system of chickens of any age, but is capable of doing so, in young hosts, after a process of variation and adaptation to another species, the duck. The varied virus can then be compared to other viruses, such as those of the Eastern and Western encephalitis,

which affect the central nervous system exclusively or preferably in young animals. Yet, one would hesitate to call the duck virus neurotropic if this expression conveys the idea of an effect on the nervous tissue proper because, as far as our study goes, only the vascular system was observed as being directly affected by the virus. Possibly strengthening this view is the fact that intracranial injections of the duck virus were little effective in ducklings, large amounts of virus being needed to induce lesions (mostly sarcomas) which developed in the meninges. Conceivably, in this way of inoculation the virus gained access to much fewer vessels than when it infects the nervous tissue from the blood stream. Contrasting with this, the virus of the original Rous virus never induced lesions of the central nervous system of chicks when circulating in the blood, but it did almost constantly induce tumors in the meninges and vessels when injected intracerebrally.

Two more facts deserve mention in this connection. One is that the chick, a host susceptible to variant 14(d)7, never showed lesions in the central nervous system after intravenous injection or as a generalization of primary tumors induced by this variant. The other is that ducklings proved to be entirely resistant to the intracranial injection of the original Rous sarcoma virus. All this emphasizes the subtleness of the process of virus variation manifested by the different reactions of the same tissue to different virus variants injected by different routes.

The second point of interest in our study concerns the analogies between some of the vascular lesions induced by duck tumor virus in the central nervous system of ducklings and the lesions induced in the same structures by so many ordinary viruses.[1, 11, 12, 14] The analogies are unusually striking with the lesions in the central nervous system described by Green *et al.*[1, 12] in fox encephalitis. This is especially true considering that in the latter disease the nerve cells do not appear to be directly involved, the most consistent lesions being those of the vascular endothelium; also, in that hemorrhage was commonly found in many locations other than the brain. All this emphasizes once more that the hemorrhagic lesions induced by tumor viruses are the result of a direct attack on the vascular cells by the virus and are not an indirect effect of tumor growth.[4, 15]

One naturally wonders what the evolution of these lesions would have been had the host lived long enough. Possibly some of the endothelial proliferations would have become progressively growing sarcomas like those developing in the viscera. On the other hand some of the thickened vessels also showed necrotic phenomena specially in the medial layer. It may be that endothelial and adventitial stimulation followed that initial medial

necrosis, but as in some cases massive necrosis was observed, one must consider the possibility that the stimulated intimal and adventitial cells, in turn, underwent necrosis at a later phase. Then, the "cancer" virus would have behaved like so many ordinary viruses which destroy the infected cells after an initial stimulation.

In conclusion, it has been shown that in a young host the lesions induced by a "cancer" virus in a particular tissue, the vessels of the central nervous system, are morphologically similar to and may have followed the same course as the lesions induced by several ordinary viruses in the same tissue. When the host has grown older and consequently more resistant, then no lesions of any type develop in this tissue, although they may in others.

Summary

The virus of a duck variant of the Rous sarcoma of chickens, strain 14(d)7, was found to be endowed with the property, unique in tumor viruses, of inducing lesions in the vessels of the central nervous system of young ducks—never in older individuals—when circulating in the blood stream.

This contrasts with the behavior of the original Rous virus which never affects the central nervous system under the same conditions.

Grossly, these lesions in ducklings were always hemorrhagic. Microscopically, some of them showed in addition to hemorrhage pronounced changes in the vessel walls consisting of medial necrosis together with intimal and adventitial proliferation or massive necrosis of the entire wall, the whole process being very similar to that observed in infection by ordinary viruses. On the other hand, a few of the proliferative lesions evolved into a minute foci consisting of cells very much resembling sarcoma cells.

The virus of the duck variant injected intracranially into ducklings induced only minimal vascular lesions; by considerably increasing the amount of the inoculum it sometimes induced sarcomas in the meninges, mostly arising from vessels. In contrast with this, the original Rous virus similarly injected into chicks induced sarcomas almost constantly.

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POST-MORTEM PULMONARY EDEMA*

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It is especially appropriate that a paper originating from the Medical Division of the Army Chemical Center be included in this anniversary volume of the *Yale Journal of Biology and Medicine*. Dr. Winternitz has been actively connected with chemical warfare medical research from the beginning and particularly during the two wartime periods of greatest activity. During the war against the Central Powers he was concerned with determination of the anatomical changes produced by the various chemical warfare agents. The summary of that work, published in 1920,[1] still serves as a standard description of the lesions. During the recent war, as Chairman of the Committee on Treatment of Gas Casualties of the National Research Council, he supervised the activities of many teams of investigators. It was in great part through his efforts in this capacity that the plans for the Medical Division, Army Chemical Center, were initiated and carried through. The problem of the mechanism of formation of pulmonary edema was acutely brought to the fore by the early studies of chemical warfare agents, particularly chlorine and phosgene. After the first world war Dr. Winternitz made important contributions to the field,[2,3,4,5] and his interest in this ever-present enigmatic subject persists. It is doubly fitting that a paper on the mechanism of formation of pulmonary edema be included in this volume.

In the evaluation of pulmonary edema in experimental animals following exposure to irritant gases, it has been observed that animals examined at intervals after death exhibit more extensive pulmonary edema than similar animals examined immediately. This observation has led to hesitancy in the interpretation of pulmonary findings in animals that succumb when the laboratory is unattended and autopsy is delayed. In sacrificed animals, the effect of the method of sacrifice on the pulmonary findings also required evaluation. The following experiments were performed with the object of determining the influence of the method of sacrifice and of the interval after death on the condition of the lungs at autopsy.

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Methods

Dutch and albino rabbits that had recovered for at least three weeks from skin or eye applications of various toxic chemicals were sacrificed by the following methods:

Air embolism. An intravenous injection of 3 cc. of air per kilogram of body weight was made as rapidly as possible through a #21 gauge needle.

Nembutal. Abbott's Veterinary Nembutal containing 60 mg. per cc. was used for all injections. The injections were made as rapidly as possible through a #21 gauge needle and death occurred promptly. One group of animals were anesthetized slowly with 60 mg. per kg. at the rate of 1 cc. per minute. Respirations ceased in the latter group approximately one minute after injection was completed.

Ether. A cotton pledget soaked with ether was placed in a cone-shaped mask. The animals were anesthetized with open drop ether until respirations ceased. The time of death varied from 5 to 13 minutes.

Electrocution. A 110-volt alternating current was passed through the body with electrodes on the lip and in the rectum. The current was applied for 45 seconds. One group of animals received heparin prior to electrocution.

Exsanguination. In some instances, blood was removed through a needle inserted into the left ventricle; in others, exsanguination was achieved by severing the femoral arteries and veins.

Rabbit punch. The rabbit was suspended by the hind-legs and a sharp blow was administered to the back of the head with the ulnar surface of the hand.

Magnesium sulphate. A saturated solution of magnesium sulphate was administered as rapidly as possible in doses of 1.5 cc. per kg. through a #21 gauge needle.

Procedures. When injections were to be made into the chambers of the heart, the rabbit was tied on its back and the needle introduced into the chest close to the sternum. Blood was withdrawn into the syringe and the position of the needle in the heart was judged by the state of oxygenation of the aspirated blood. Autopsy showed that perforations of the lung were not produced by this technique.

Animals in the groups designated "Immediate" were autopsied as soon as signs of life were no longer present. In the "Delayed" groups, unless otherwise specified, the rabbits (placed on their abdomens with the legs extended) remained at room temperature for the designated time. A few experiments were conducted at 35°F. In some "Delayed" groups the trachea was clamped or a cannula was inserted into the trachea immediately after respiration ceased. The effect of positive air pressure upon the formation of edema was tested by connecting the tracheal cannula to air at 10 cm. of water pressure or at 30 cm. of water pressure for a three-hour period.

In experiments designed to determine the distribution of injected Nembutal within the animal, a small amount of solution containing phosphorous[32] in the form of sodium phosphate was added to the Nembutal. Samples of blood and tissues taken from various portions of the body after injection were tested for radioactivity by a Beckman MX5 survey meter.

Method of examination. At the time of examination, an incision was made into the neck and the trachea was clamped 1 cm. below the thyroid cartilage. The chest plate was removed and the lungs, trachea, bronchi, esophagus, and thymus were removed *en bloc.* The lungs were dissected clean and the trachea was ligated at the level of the carina and severed immediately above the tie. The lungs were examined grossly,

weighed, and immediately submerged in 10% formalin. After hardening for twenty-four hours, thin sections were removed from the right lower lobe and left upper lobe and were fixed in 10% formalin for microscopic sections. The lung weight to body weight ratio was obtained by dividing the lung weight in grams by the body weight in kilograms. The mean and the standard error of the mean for each group were determined. Comparisons between the groups were made by the application of the t test. The P value for such a comparison was taken from standard tables. Significant values for P were .05 or lower.

Results

Immediate examination—gross findings. In animals examined immediately after death the lungs were uniformly light, air containing, and free of gross evidence of edema, regardless of the method of sacrifice. Following exsanguination the lungs were exceedingly pale, while after ether, the pulmonary surfaces appeared pale and cyanotic. Petechial hemorrhages under the pleura and throughout the parenchyma resulted from electrocution. In the groups sacrificed with Nembutal, magnesium sulphate, or the rabbit punch, congestion of the lungs was more marked than in those sacrificed with air. The lung weight to body weight ratios did not differ greatly in any of the groups examined immediately after death (see Table 1, columns 2 and 3). The lowest ratios were obtained in the ether ($3.23 \pm .24$) and the exsanguination groups ($3.57 \pm .21$). Several groups sacrificed with Nembutal had ratios greater than 4.0.

Immediate examination—microscopic findings. The capillaries of the alveolar walls of animals sacrificed by exsanguination or ether were devoid of blood. In contrast, more congestion of the alveolar walls followed Nembutal, magnesium sulphate, and rabbit punch. Small hemorrhages were noted within the alveoli in the electrocuted, air, and rabbit punch groups. Following the rabbit punch, bone marrow emboli were present in the large branches of the pulmonary arteries. In the groups sacrificed by Nembutal and having the highest lung weight to body weight ratio a minute amount of fluid was present in the alveoli in small perivascular foci.

Delayed examination—gross findings. The lungs of all animals except those of the exsanguination group examined three hours after sacrifice were congested and edematous. These findings varied from small patchy areas of edema or generalized increased turgidity in the animals sacrificed with air, electrocution, or the rabbit punch to almost complete involvement of all lobes by dark, currant jelly, boggy consolidation following ether, magnesium sulphate, or Nembutal. The exsanguination group showed no evidence of congestion or edema after three hours. In the other groups the extent of edema paralleled the ratio of the lung weight to body weight (Table 1,

TABLE 1

EFFECT OF METHOD OF SACRIFICE ON LUNG WEIGHT

Method of sacrifice and treatment	Immediate autopsy		Autopsy after 3 hours		t value	P
	No. of animals	Lung weight per kilo ± S.E. mean	No. of animals	Lung weight per kilo ± S.E. mean		
		Grams		Grams		
Exsanguination	5	3.57 ± .21	6	3.62 ± .13	2.138	>.05
Open drop ether	5	3.23 ± .24	10	7.42 ± .73	3.918	.01
Air (3 cc./kg.) i.v.	5	3.65 ± .18	5	4.81 ± .31	3.213	.01
Air (3 cc./kg.) i.v.—open cannula	..	.....	5	5.14 ± .61	2.330	.05
Air (3 cc./kg.) i.v.—trachea clamped	..	.....	5	5.51 ± .33	4.950	.001
Electrocution	5	3.91 ± .45	5	5.80 ± .34	3.323	.01
Heparin, then electrocution	..	.....	5	5.24 ± .28	....	....
Rabbit punch	10	3.85 ± .20	11	6.68 ± .42	5.855	.001
$MgSO_4$ (1.5 cc./kg.) R. ventricle	5	3.24 ± .31	5	9.77 ± .46	11.736	.001
$MgSO_4$ (1.5 cc./kg.) L. ventricle	5	3.86 ± .25	5	8.21 ± .95	4.425	.01
Nembutal (100 mg./kg.) i.p.	5	3.69 ± .25	5	5.98 ± .60	3.491	.01
Nembutal (60 mg./kg.) i.v.—slow	5	4.04 ± .11	5	5.33 ± .84	1.532	>.05
Nembutal (60 mg./kg.) i.v.—rapid	5	4.27 ± .38	5	6.04 ± .51	2.776	.05
Nembutal (100 mg./kg.) i.v.	5	3.83 ± .27	5	9.47 ± .74	7.116	.001
Nembutal (100 mg./kg.) i.v.—trachea clamped	..	.....	6	9.35 ± .77	6.246	.001
Nembutal (100 mg./kg.) i.v.—open cannula	..	.....	19	9.46 ± .62	4.617	.001
Nembutal (100 mg./kg.) i.v.—kept at 35°F.	..	.....	15	6.96 ± .62	2.821	.01
Nembutal (100 mg./kg.) i.v.—10 cm. water*	..	.....	6	7.43 ± .49	6.121	.001
Nembutal (100 mg./kg.) i.v.—30 cm. water*	..	.....	6	4.82 ± .23	2.835	.05
Nembutal (200 mg./kg.) i.v.—open cannula	..	.....	8	13.54 ± 1.26	....	....
Nembutal (100 mg./kg.) L. ventricle	5	4.01 ± .16	10	8.80 ± .75	4.426	.001
Nembutal (100 mg./kg.) R. ventricle	5	4.57 ± .37	10	12.14 ± 1.06	4.893	.001

* Positive air pressure applied through tracheal cannula.

columns 4 and 5). The highest values were obtained with Nembutal or magnesium sulphate, particularly when introduced into the right ventricle. The degree of edema following Nembutal varied directly with the quantity injected. There was no significant difference in the appearance of the lungs or in the lung weights in the groups given 100 mg. per kilogram of Nembutal or air injected intravenously whether the trachea was clamped, opened to the atmosphere by insertion of a cannula, or untouched during the three-hour post-mortem period. Application of positive tracheal pressure suppressed the formation of edema; the lung weights varied inversely with the magnitude of the pressure. Maintenance of Nembutal-sacrificed animals at 35° F. during the three-hour period diminished the amount of edema formed. Administration of heparin prior to electrocution did not change the lung weight appreciably. Intravenous administration of 100 mg. per kilogram of Nembutal produced greater pulmonary edema than intraperitoneal or left ventricular injection but less change than injection into the right ventricle.

Delayed examination—microscopic findings. In the exsanguination group there was no evidence of change after three hours (Fig. 1, a and b). Capillary congestion was not prominent in the animals sacrificed with air, but there were patchy areas of edema involving alveoli and bronchioles (Fig. 2, a and b). Following the other methods of sacrifice, congestion and edema were more extensive and the degree of involvement paralleled the ratio of the lung weight to the body weight (Fig. 3, a and b, and Fig. 4, a and b). In the most severe instances almost all alveolar space was filled with homogeneous pink-staining material (Fig. 5, a and b).

Comparison of immediate and delayed groups. The results of comparison of the lung weights of the immediate and the delayed groups sacrificed by the various methods are presented in Table 1, columns 6 and 7. Where special procedures were performed during the interval after death (tracheal clamp, positive pressure, etc.), the comparison was made with the group of animals sacrificed by the same method and examined immediately. In two instances a statistical analysis was not made because suitable controls were lacking. The lung weights after three hours were significantly increased over the corresponding immediate controls in all instances except following exsanguination and after slow injection of 60 mg. per kilogram of Nembutal.

Effect of the interval after death. A few experiments were performed to determine the effect of the length of the post-mortem interval upon the

quantity of edema. The results of these experiments are presented in Table 2. The lung weight reached a maximum before four hours after sacrifice.

Extent of circulation after injection of Nembutal into left ventricle. Because of the unexpected severity of the pulmonary edema following introduction of 100 mg. per kilogram of Nembutal into the left ventricle, it became of interest to determine the extent of the circulation after such an injection. Death ensues within a few seconds; rarely is there any respiratory effort, and heart action ceases promptly. The following experiment was made to

TABLE 2

EFFECT OF INTERVAL AFTER SACRIFICE BY NEMBUTAL (100 MG./KG.) ON LUNG WEIGHT

Interval after sacrifice	*Treatment*	*Number of animals*	*Lung weight per kilo* $\pm$ *S.E.* $_{mean}$
			Grams
Immediate		5	3.83 ± .27
1 hours	Cannula in trachea	5	5.42 ± .58
2 hours	Cannula in trachea	5	7.09 ± 1.39
3 hours	Cannula in trachea	19	9.46 ± .62
4 hours	Cannula in trachea	5	10.88 ± 1.53
6 hours	Cannula in trachea	5	10.95 ± .74

determine whether under such circumstances the injected material reached the lung.

A rabbit was sacrificed by left ventricular injection of Nembutal (100 mg. per kilogram) in 6.8 cc. of solution containing phosphorous[32] in the form of sodium phosphate (total activity of injected material: 23.8 m. rep.). Death was instantaneous. Clamps were immediately placed on the carotid arteries, trachea, the great vessels at the base of the heart, the hilum of each lung, and the aorta at the level of the diaphragm. Samples of blood and tissue from various sites were removed and tested for radioactivity. Only background activity was recorded from blood removed from the right ventricle, cerebral cortex, liver, and intestine. Radioactivity in excess of background was obtained from blood from the left ventricle, the thoracic aorta, the abdominal aorta, spleen, kidney, and all lobes of the lungs. Greater activity was present in the lower than in the upper lobes. It was concluded that despite the rapidity of death following introduction of Nembutal into the left ventricle, the injected material had reached the lungs.

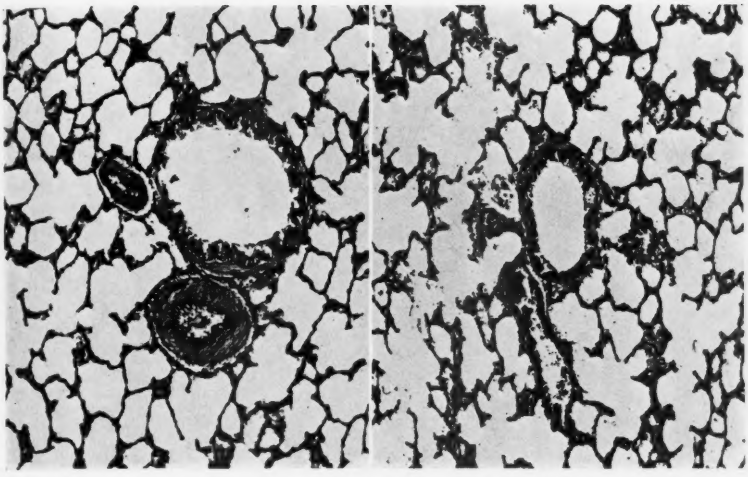

FIG. 1. Lungs of rabbits sacrificed by exsanguination: a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

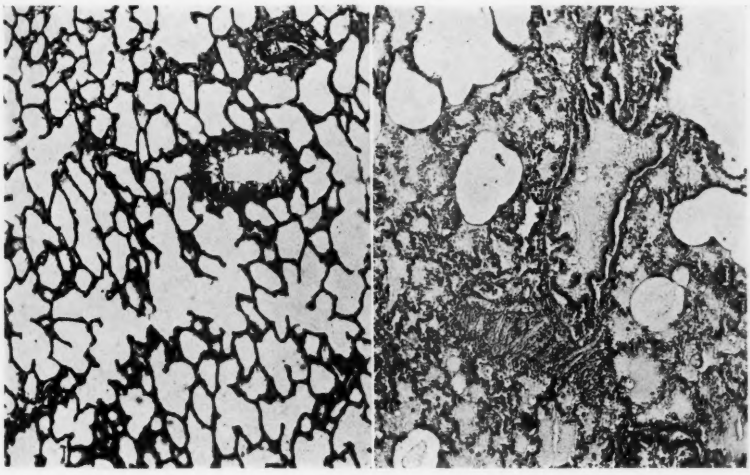

FIG. 2. Lungs of rabbits sacrificed by intravenous injection of air: a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

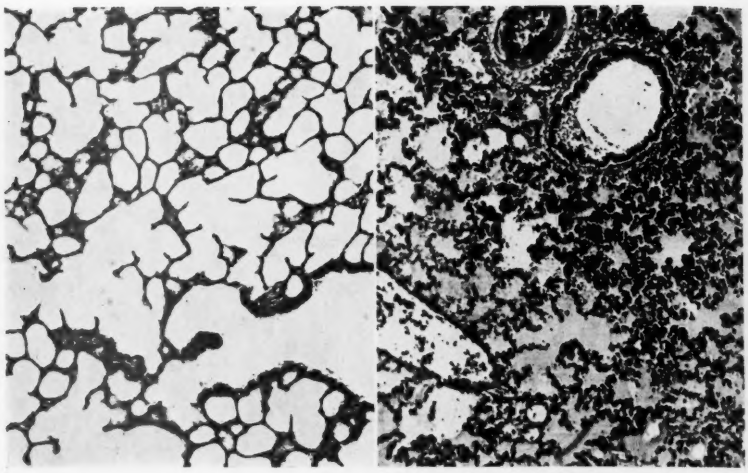

FIG. 3. Lungs of rabbits sacrificed by rabbit punch: a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

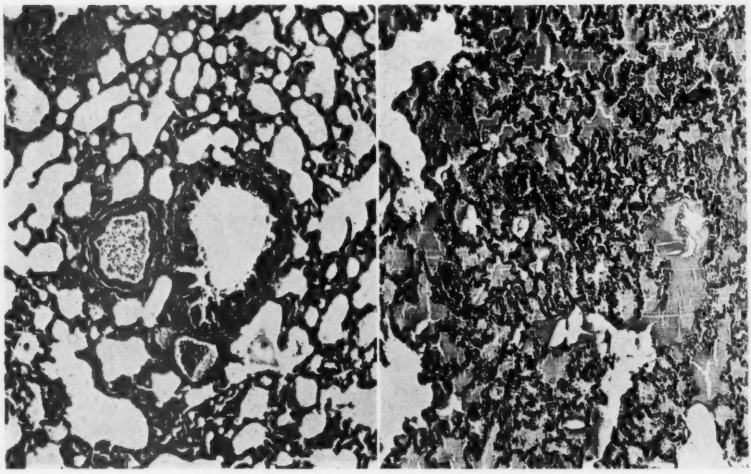

FIG. 4. Lungs of rabbits sacrificed by intravenous injection of Nembutal (100 mg. per kilogram): a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

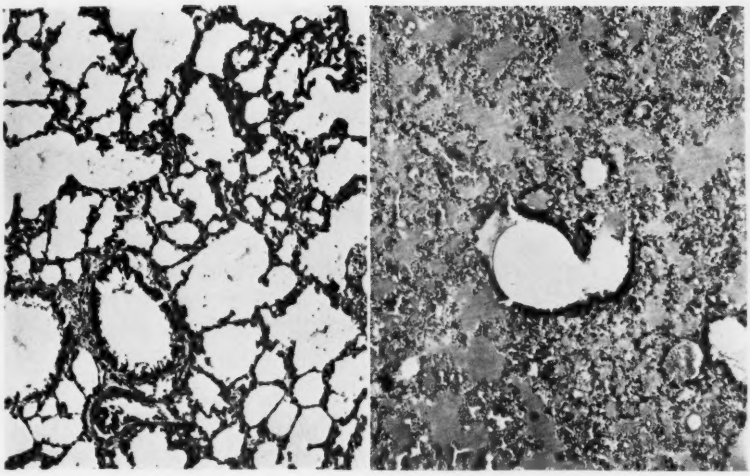

FIG. 5. Lungs of rabbits sacrificed by right ventricular injection of Nembutal (100 mg. per kilogram) : a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

Discussion

Pulmonary edema that cannot be differentiated by its gross or microscopic appearance from that produced by many noxious agents develops in animals during the post-mortem period. It is not possible to evaluate the lung findings in experimental animals unless examination is performed immediately after death. Autopsy in humans is usually delayed several hours and pulmonary edema and congestion are almost constant findings. The experiments reported here suggest that in many instances such pulmonary findings in human cases may result from post-mortem change.

Two of the factors responsible for the formation of pulmonary edema in the post-mortem period and brought out by these experiments are a pressure gradient between pulmonary vasculature and the alveolar spaces and an alteration of capillary permeability. It is apparent that a gradient of pressure must exist between the pulmonary vasculature and the alveolar spaces for edema to occur. Exsanguination with its concomitant lowering of intravascular pressure prevents formation of edema. Positive air pressure of 10 cm. of water applied to the alveoli diminishes the amount of edema. A pressure of 30 cm. of water effectively prevented edema.

The quantity of edema varies with the dose and route of administration of the Nembutal. It appears that the amount of edema parallels the concentration of Nembutal in the pulmonary capillaries and that Nembutal has a direct action on the permeability of these structures. Intravenous administration of 100 mg. per kilogram of Nembutal results in more edema than intraperitoneal injection of the same quantity but causes less edema than the introduction of the same amount of material into the right ventricle. Left ventricular administration causes less edema than intravenous or right ventricular injection. Experiments with radioactive material injected into the left ventricle indicate that some injected material reaches the lungs via the bronchial arteries or by reverse flow through the pulmonary veins or both.

Summary and conclusion

Pulmonary congestion and edema develop during the first few hours of the post-mortem period in rabbits sacrificed by a variety of methods including: Nembutal, magnesium sulphate, intravenous air, electrocution, rabbit punch, and the inhalation of ether. Following exsanguination post-mortem pulmonary edema does not occur. The quantity of edema present three hours after death varies with the method of sacrifice, the route of administration of the lethal agent, the temperature, and the intratracheal

pressure. Evaluation of pulmonary edema and congestion should not be made in experimental animals unless examination is made immediately after death.

Acknowledgment is made of the valuable technical assistance of Mr. Andrew J. Miller and Mr. Arthur Fisk.

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REMINISCENCES OF AN ERSTWHILE STUDENT

LUCILLE R. FARQUHAR

In 1933 the Dean interviewed applicants for the Yale Medical School from the depths of a big chair at an enormous shiny table. I can't remember any thing about the room—probably because I never took my eyes off the man in the chair. Rumor had painted this interview as a shattering affair. Although I had been I.Q.ed, testimonialed, and bolstered with academic records, I was nervous and numb. I knew Dr. Winternitz only by sight. We started routinely enough—name, age, previous condition of servitude. We then talked a few minutes during which he suggested that I was getting on in years for new adventures into learning and, after I had seen the psychiatrist (who might or might not consider me insane to attempt such a venture), he would consider my chances. I left him wondering if I was sane after all.

A year later we fledglings crossed the street to get our noses rubbed into diseases, their causes and effects. We must have taken something besides pathology and bacteriology, but only those courses stand out clearly now. The first shock of pathology was contact with the bodies. Pretty soon we were supposed to recognize a few things on slides, and before long came lectures on the kidney. Other members of the staff had lectured before, but only "Winter" could do the kidney. Left hand on the small of the back protecting his own filtration system (which seemed to distress him), glasses twirling in the right hand, he tramped the stage and roared the elemental details. It was fine. It was fun. It was a vaudeville show. It became tense when questions were popped, or quizzes. But it also became a password, "Winter is lecturing on the kidney!" We enjoyed the show but made few notes. Out of class, the illusion of having learned something passed. We consulted references in the library.

At the weekly, or biweekly, session with organs, slides, and questions some unfortunate would give as his opinion a quotation from Boyd or Karsner, perhaps a neat definition of subacute nephritis. Winter would hear him out, ask him to explain what he expected to find in the gross and on the slide, and what he saw in the half kidney dripping formaldehyde from the professor's hand. As the student floundered deeper and deeper into hell with red ears and halting speech, Winter would goad him on from

absurdity to absurdity while we all trembled. The final annihilating blast eventually allowed him to collapse into the relative comfort of anonymity on a chair in the row with his classmates. Then it was the next man's turn to put up or take it. Anything that smacked of honest observation or logical thinking would save you from the storm, but quoting another man's words, undigested and spewed forth, was tantamount to suicide. And once you had blundered, you could be sure of an opportunity to repeat your agony. Nobody slept. Perspiration dripped in midwinter. We learned to think it out, observe, reason, talk on our feet, or suffer Winter's scorn. Some of us found it stimulating, others swore such cruelty had not been abroad since the Romans threw the martyrs to the lions. But pathology remained a "top interest" course on which we spent about fifty per cent of our time; and most of us were *for* Winter even though he scared the "daylights" out of us. I can still feel my adrenals at work when I remember those sessions. Yet, the girls in the class were relatively well treated. Chivalry? Or you can't expect too much of women anyhow? Ask Winter. Perhaps you'll get a straight answer, perhaps a conundrum. It is sure to be interesting either way.

MILTON CHARLES WINTERNITZ

ABRAHAM FLEXNER

I do not remember my first meeting with Winternitz, but, when the full-time clinical experiment was launched in Baltimore, Winternitz was one of my first customers. He was and still is one of our ablest medical scientists.

In his account of *William Henry Welch and the Heroic Age of American Medicine* my brother, Simon, speaks of a group which included such brilliant men as MacCallum, Opie, G. H. Whipple, and Winternitz. "These men," he said, "and many others who also made important contributions, carried Welch's influence all over the nation, until American pathology came to equal and even perhaps exceed the European schools on which it was modelled."

Winternitz—or, as he is always called by his friends, "Winter"—was born in Baltimore of poor, but intelligent parents who sacrificed everything to educate their son. As he grew up, he mixed freely with all kinds of people, including (in his own words) the elegant of Eutaw Place.

He received his B.A. degree from Johns Hopkins in 1903 and his doctorate in medicine in 1907. Among his teachers in the University, all of whom were impressed with his ability and character, were Brooks, the biologist, and Remsen, the chemist. In the Medical School, Welch, Mall, Howell, Abel, and Osler inspired him with the enthusiasm which has been characteristic of his whole scientific life. In 1917, Dr. Welch, answering a call from the Yale authorities for a new pathologist and bacteriologist, suggested Winternitz. Three years later he became dean of the Medical School, a post which he held until 1935.

Between 1919 and 1921 Mr. Rockefeller, Senior, presented the General Education Board with almost $50,000,000 to be spent, principal and interest, for the improvement of medical education in the United States. Winternitz was among the first and most persistent of those who sat on my doorstep.

In an autobiography called *I Remember,* published in 1940, I said of Winternitz that he was one of the most energetic, keen, and forceful administrators that I encountered in the whole course of my dealings with medical schools. The phenomenal rise of the Yale Medical School is an interesting story. No sooner was full-time teaching introduced into the

Johns Hopkins than Winternitz posted down to New York to see me. It was obvious that the school had simultaneously to raise endowment, to find a building fund, and to reorganize its faculty. At the request of Winternitz I went to New Haven and visited the Medical School and the New Haven Hospital with him. The shortcomings were enough to daunt anyone but Winternitz himself. As a matter of courtesy, of course, I first called on President Hadley, who was skeptical as to the possibility of raising funds or making the necessary changes in personnel without creating too great a furor. I recall very distinctly this interview with President Hadley in his room in Woodbridge Hall. In his nervous way he paced up and down, explaining to me that before I went into details with Winternitz he wished to call to my attention a few points which he thought would have to be borne in mind. He said:

"The Yale Medical School is not only practically without funds but relies upon the facilities and courtesies of the New Haven Hospital, which we have hitherto enjoyed free of all cost. Now, the head of the Board of the New Haven Hospital is Colonel Isaac Ullman, and it is in reference to Colonel Ullman that I particularly want to speak to you. In the first place, Colonel Ullman is not a Yale graduate."

"Well," I replied, "inasmuch as I am not a Yale graduate myself, I do not think we are likely to encounter difficulties on that score."

"In the second place," he said, "Colonel Ullman's closest friend is a Roman Catholic while he himself is a Jew, and we are anxious to avoid anything that might be attributed to racial and religious prejudice."

I answered, "As I am a Jew myself and have been for years making my way among Christians and working with them, I think the situation can be handled tactfully, so that prejudice need not be stirred."

"Finally," said Mr. Hadley, "there is a third point, namely, Colonel Ullman's business. He is a corset manufacturer."

"Oh well," I said, "now I feel perfectly certain that he and I will get on famously."

I left President Hadley and went to the Graduate Club, where I met Colonel Ullman and Winternitz. Colonel Ullman was a shrewd bargainer.

"How much is this structure going to cost the New Haven Hospital?"

"It will not require a big sum to begin with, but in the course of time the whole hospital will have to be reconstructed and reorganized. That can be made a matter of years, the speed depending upon the pride of the citizens of New Haven in the rejuvenated institution."

"Well," said Colonel Ullman, "can we do anything with three hundred thousand dollars?"

"Oh yes," said I gaily, "with that sum Winternitz and his associates can do a good deal."

"How much will the General Education Board contribute?"

I mentioned a sum which left it incumbent upon citizens of New Haven to raise one half. Naturally Ullman wanted more, but I pointed out to him that, if the school was to be reorganized, the main financial support would have to come from New Haven and the alumni of Yale, since it was to be the Yale Medical School and not the General Education Board Medical School. Ullman agreed.

"I will," he said, "give a dinner at the Hotel Taft, and I will get Bill Taft to come up and preside. Will you come to New Haven and make a speech?"

I replied that I was an inexperienced speaker but that I would do anything to help the good cause.

"Very well," replied Ullman, "what would you say?"

I had no time for thought, and I did not know the local situation, but on the spur of the moment I suggested a line which I might take. With the candor that was characteristic of our relations as long as the Colonel lived, Ullman replied: "If you make that speech, we won't get a cent."

"Very well," I said, "I can make a different kind of speech," and then quickly outlined a quite different kind of address.

"Fine," said the Colonel, "if you will talk that way, we will get the money."

The dinner was held, and about one hundred citizens of New Haven attended. Colonel Ullman was in great form, exuberant in his enthusiasm, and really showed that he had caught the vision which Winternitz had been dangling before him. Taft presided with his accustomed geniality. The table reverberated with laughter, and Taft's chuckle, as a sally of some sort or other was made here and there, kept the merriment going. When the time for speechmaking came, I fulfilled my bargain. Winternitz and Stokes spoke seriously and well. The sum which Ullman set out to raise locally was procured in a relatively brief time.

This volume is published to honor Winternitz. Is there any doubt in the mind of the reader that I have placed on his head the myrtle crown of victory with which the Romans decked their successful warriors? It detracts nothing from his laurels if I beg you to remember at this time the scientists by whom Winter was trained, the great University where he was trained, and the wise and generous philanthropists to whose unstinted generosity the Johns Hopkins University owes its being—Johns Hopkins himself, President Gilman, the two Rockefellers, father and son, and finally Mr. Frederick T. Gates and Dr. Wallace Buttrick.

THE RELATION OF HERPES VIRUS TO THE CELL NUCLEUS*

THOMAS FRANCIS, JR. AND HILDA B. KURTZ

The selectivity with which viruses tend to attack specific tissues and cells, resulting in characteristic types of morphologic alteration, readily invites investigation of the manner in which the virus becomes attached to the cell. Infection with the virus of herpes simplex is typified by the presence of inclusion bodies in the nucleus which have been thought by some investigators to represent the localized virus. This possibility is well discussed in van Rooyen and Rhodes.[17]

In recent years increasing use has been made of centrifugation as a method for separating the structural units of cells.[5] It was thought, then, that similar procedures could be employed to determine whether herpes virus has a selective affinity for the nuclei of susceptible cells and to ascertain whether the virus is intimately related to the nucleoprotein of the nucleus.

The present paper is in reality a progress report including a description of the experimental approach and an interpretation of the data which have been obtained.

Materials and methods

1. *Preparation of virus.* Two different strains of herpes simplex virus were used. The J. R. Smith strain was maintained through seven passages in mouse brain after being received in our laboratory from Dr. J. E. Smadel of the Army Medical Department Research and Graduate School, Army Medical Center. Eight mouse brains, removed three days after infection, were ground and made into a 10 per cent suspension in 10 per cent horse serum-saline. After centrifugation at 1500 rpm for 20 minutes, 3-4 cc. were placed in small ampules which were sealed with heat and frozen in a dry ice cabinet. Culture of the suspension was negative for bacteria after 48 hours. Titration, done shortly after freezing, by intracerebral inocultion of mice showed a 50 per cent end point[14] of $10^{-4.6}$. Based upon this evaluation approximately 1000 LD50's were employed in the tests. It can be pointed out, however, that the virus is not uniformly stable under these conditions of storage and variation in the actual amount of virus resulted.

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* From the Department of Epidemiology and Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor, Michigan. Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

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The Armstrong strain of virus was received from Dr. Charles Armstrong of the National Institutes of Health as mouse brain from the forty-eighth passage. It was adapted by repeated passage on the chorio-allantoic membrane of the embryonate egg until it produced gross lesions in the liver and heart of the embryonic chick.[3] Pools of virus were prepared from infected membranes of the twenty-fifth or later passage collected on the third or fourth day after inoculation of 10-12-day embryos. The membranes were ground, and 10 per cent suspensions were made in 10 per cent horse serum-saline. After centrifugation, the supernatant was sealed in glass ampules and frozen. Tests for sterility were made, and the titer of virus then determined by intracerebral inoculation of mice. The titers attained by the twenty-fifth and twenty-eighth passage material were, respectively, $10^{-4.83}$ and $10^{-4.75}$. Sometime after this the virus appeared to be losing its virulence for mice and consequently was passed through the brains of mice for five passages and then transferred back to eggs. Pools from the second and the sixth subsequent passages in eggs had titers in mice of $10^{-5.5}$.

The titrations were done by intracerebral inoculation of groups of six mice which were observed for 21 days and the fatalities recorded. The LD50 was calculated upon deaths in the first 14 days, since after that time only occasional irregular deaths were observed.

2. *Preparation of cell fractions from embryonic chick liver:* (a) *In hypotonic salt solution.* This procedure for obtaining nuclei is similar to that described by Hoerr[9] as a modification of Dounce's procedure.[8] All containers, apparatus, and reagents were chilled, and all operations were so far as possible carried out in a cold room. All centrifugations were performed in the cold (—6°C).

Livers of 14-15-day embryos were collected aseptically in a petri dish after allowing the embryos to bleed freely. The livers were chilled immediately in an ice bath. They were then rinsed in isotonic salt solution to wash off adhering blood, drained, and minced finely with curved scissors. Working in a refrigerated room, the minced livers were ground lightly in small amounts in small mortars, adding gradually 0.6 per cent NaCl solution buffered to pH 6.1 with 0.01M PO_4. A final concentration of 1.0 gm. of liver to 5.0 cc. of solution was prepared, the suspension was strained through four layers of gauze, and the fluid portion was centrifuged for 20 minutes at 2000 rpm in a horizontal centrifuge. The supernatant fluid was carefully decanted and the sediment, containing nuclei, red cells, liver cells, and debris, was resuspended in the original volume of hypotonic saline, taking care to stir gently so as not to rupture the nuclear membranes. The material was centrifuged for 15 minutes at 1500 rpm and the supernatant poured off. The residue was again resuspended in the original volume of saline and centrifuged for 10 minutes at 1500 rpm. The sedimented nuclei were then resuspended in one half the volume and centrifuged 5 minutes at 1000 rpm. The supernatant was discarded, the residue resuspended again in one half volume and centrifuged for 3 minutes at 800 rpm; this was done three times. The nuclei were then suspended in the original volume of hypotonic saline and allowed to settle in a tall cylinder for 45 minutes in the cold. The supernatant, containing the nuclei, was carefully removed by pipette and the nuclei recovered by centrifugation for 3 minutes at 800 rpm. The volume of packed sediment was noted and a 10 per cent suspension was prepared in 10 per cent horse serum-saline. The number of nuclei in a preparation was then determined by the addition of methyl green and counting in a hemacytometer. On the average, approximately 31,000 nuclei were counted per cu. mm. A suspension

for comparison was prepared by grinding whole embryonic livers thoroughly in mortars so as to break cellular structures and to free the component materials. A 20 per cent suspension was made in 0.6 per cent NaCl solution pH 6.1. The suspension was centrifuged at 1000 rpm for 1 minute, the supernatant collected and recentrifuged at 3000 rpm for 5 minutes. The sediment was resuspended in 10 per cent horse serum-saline to form a 10 per cent suspension and used in this form.

(b) *In sucrose.* This procedure which was used primarily for fractionation of infected livers is an extension of that described by Hogeboom, Schneider, and Pallade[10] for the preparaiton of mitochondria. It was carried out with sterile technic at 4°C. After bleeding, the livers are removed from 15-day chick embryos 4 days after infection with Armstrong strain virus and placed in cold 0.85 per cent NaCl. They are then drained on gauze and weighed. They are minced with scissors, a 20 per cent suspension prepared in 30 per cent (0.88M) sucrose, and homogenized in a glass homogenizer[13] for 2 minutes at approximately 1000 rpm. The material is strained through four layers of gauze and the gauze washed with sufficient sucrose to regain the original volume. One half cc. volumes of sucrose are placed in graduated centrifuge tubes and the homogenate is carefully introduced so as to prevent mixing. The tubes are centrifuged at 1800 rpm for 20 minutes in a horizontal centrifuge. The supernatant is then carefully removed and serves as the source of mitochondria and microsomes. The sediment contains the hepatic nuclei, some whole hepatic cells, erythrocytes, and debris; its volume is noted in the graduated tube.

After straining through gauze an aliquot of the homogenate was removed and ground with alundum, suspended to 10 per cent in 30 per cent sucrose and centrifuged at 1800 rpm for 10 minutes. The supernatant was titrated and served as the virus control.

Mitochondria and microsomes. To remove any remaining nuclei the supernatant is centrifuged at 1800 rpm for 10 minutes three more times, the fluid being poured into a fresh tube each time. The final supernatant is centrifuged at 16,000 rpm for 20 minutes in an anglehead centrifuge and the fluid portion poured off and saved. This is referred to as the 20 per cent suspension of microsomes. No attempt to purify the microsomes is made since the speed of centrifugation required would also sediment the virus particles. The sediment is resuspended to the original volume in sucrose and recentrifuged at 16,000 rpm for 20 minutes. The supernatant is discarded and the sediment resuspended to the orignal volume as the 20 per cent suspension of mitochondria.

Nuclei. The sediment containing the nuclei is resuspended in 30 per cent sucrose, avoiding the red blood cells which have packed first at the bottom of the tube, and the suspension centrifuged at 1800 rpm for 15 minutes. This is done four times, the sediment being resuspended in fresh sucrose solution each time. The last centrifugation is done in graduated centrifuge tubes, and the amount of sediment noted. The 20 per cent suspension of nuclei is made by suspending the last sediment in a volume of sucrose corrected for amount of sediment lost during the process.

3. *Nucleoprotein extraction.* Desoxyribosenucleoprotein (DNP) was extracted from the nuclei of liver cells following the procedure of Mirsky and Pollister.[13] Sterile technic was employed and all operations were carried out at 4°C. Livers from 15-day embryos infected with herpes virus are transferred to ice-cold 0.85 per cent NaCl, then drained on gauze and weighed. They are minced with scissors, suspended to 30 per

cent in 0.85 per cent NaCl, and homogenized for one minute in a glass homogenizer at 1000 rpm. The homogenate is centrifuged for 10 minutes at 8000 rpm in an angle-head centrifuge in the cold, and the supernatant is poured off. The tissue residue is well mixed and suspended to the original volume in 0.85 per cent NaCl and centrifuged at the same speed. This process is repeated three times and presumably removes the great bulk of cytoplasmic material including the ribonucleoprotein (RNP). The sediment of washed tissue is suspended at 40 per cent concentration, in terms of the original weight, in 0.14M NaCl and an equal volume of 2M NaCl added to give a final concentration of 1M NaCl. The mixture, which is quite viscous, is stirred rapidly over night. After 18 to 20 hours, it is centrifuged at 10,000 rpm for one hour. The viscous supernatant fluid containing the DNP is removed, its volume measured, and the nucleoprotein precipitated by pouring it into six volumes of distilled water. The precipitate is collected on a glass rod and transferred to a graduated centrifuge tube so as to measure the volume of the precipitate and the accompanying fluid. To redissolve the nucleoprotein an equal volume of 2M NaCl is added and then sufficient 1M NaCl to bring back to the volume of the extract. It is placed on the stirrer to facilitate solution. When all the material is dissolved, it is again centrifuged at 10,000 rpm for one hour and the supernatant saved. In two experiments the nucleoprotein was precipitated, dissolved, and centrifuged a second time.

The *quantitative determination* of the DNP was made as desoxyribosenucleic acid (DNA) employing the method of Stumpf[16] and the extraction procedure of Schneider[15]. In one instance, for comparison, the method of Dische[7] was used.

Experimental

The first experiments were devised to determine whether herpes virus is selectively adsorbed to nuclei of apparently susceptible cells. A volume of 4.0 cc. of a suspension of nuclei containing, on the average, approximately 31,000,000 nuclei per cc. was mixed with an equal volume of virus suspension so as to yield a final virus titer of 500-1000 LD50 per unit volume. A similar preparation of virus and the resuspended sediment of ground whole liver was made. A control of the same final concentration of virus alone in 10 per cent horse serum-saline was retained. The materials were kept at 4°C. Immediately after mixing, again 4 hours and 24 hours later, samples of the individual suspensions were removed and centrifuged at the same speeds employed in their final preparation so as to sediment the particles. The supernatant fluids were then titrated intracerebrally with fourfold dilutions to determine whether virus was removed from the fluid presumably by attachment to the available cellular material. If virus were selectively adsorbed to nuclei, it should be present in reduced amounts in the remaining supernatant fluid.

The average results of three such experiments are presented in Table 1. Two were done with J. R. Smith strain from mouse brain and one with

the Armstrong strain from chick embryo. Although there was an absence of numerical uniformity in the titers obtained, owing largely to the narrow range of virus concentration concerned, two consistent trends were noted.

TABLE 1

TESTS FOR ADSORPTION OF HERPES VIRUS BY NUCLEI

Time of sample (hrs.)	*Titer of supernatants at 4°C.**		
	Virus alone	*Virus + nuclei*	*Virus + ground liver*
0	470	335	140
4	330	245	100
24	360	270	75

* Average of 3 experiments.

The first was that the nuclei did not unite with virus to remove it in significant amounts from the suspending fluid. Secondly, the preparation of ground liver consistently reduced the amount of virus remaining in the fluid.

Although the evidence presented clearly indicates that herpes virus does not selectively combine with the nuclei separated from normal cells, the possibility remains that the virus may enter the nucleus of the whole cell by way of the cytoplasm. Efforts to demonstrate the distribution of virus in relation to different structural components were, therefore, made with nuclei, mitochondria, and microsomes of infected tissue prepared in sucrose by centrifugation (Table 2).

TABLE 2

RELATION OF VIRUS TO CELLULAR STRUCTURES OF INFECTED CELLS

	Experiment no.	
	14	*15*
Ground liver	56,000	147,000
Nuclei	32,000	25,000
Mitochondria	43,000	146,000
Microsomes	56,000	251,000

The results of two experiments are presented in Table 2. In Experiment 14 no significant difference was observed in the virus accompaniment of the different structures although that of the nuclei was the lowest. In the next

experiment, however, the nuclear preparation had a distinctly lower content of virus than the other fractions, again demonstrating that the virus was not selectively related to the cell nuclei. Interestingly, the preparations of microsomes which presumably contain the major portion of RNA[4] had the highest titers of virus.

A third type of experiment sought to determine the relation of virus activity to the DNP content of infected embryonic liver. Studies to date have shown that some viruses of animal origin contain primarily DNA.[8,11]

TABLE 3

RELATION BETWEEN DNP CONTENT AND VIRAL ACTIVITY OF EMBRYONIC LIVER INFECTED WITH HERPES VIRUS

	Experiment 25		*Experiment 26*	
		DNA		
Material	*Virus*	*γ/cc./10%*	*Virus*	*DNA*
Homogenized infected liver				
Washed with 0.14M NaCl				
First wash	562,000	23		
Fourth wash	3,200	0		
Washed tissue	40,000	250	100,000	256
Ground and centrifuged (control)				
Washed tissue				
In M/1 NaCl 4°C., 20 hours				
↓ Total mixture	316,000	305	251,000	325
Centrifugation				
↓				
Crude DNP—Supernatant	100,000	283	17,800	218
Sediment	178,000	60	43,000	31
↓				
Ppt. in 0.14M NaCl				
Redissolve in M/1 NaCl				
Centrifuged				
DNP—Supernatant	13,300	265	10,000	193
Sediment	4,000	0	4,900	7
Virus control				
Whole liver ground in M/1 NaCl				
Centrifuged 8,000 rpm — supernatant, 4°C.				
0 hours	143,000	195		
24 hours	251,000			

Furthermore, the DNP or DNA of the animal cell appears to be essentially nuclear in location. Because the nuclei constitute the major source of the cell's DNP and because herpes virus is an animal virus which might be expected to be DNA in type and which produces inclusions in the nuclei, it was thought that extraction of the DNP of infected tissue would furnish information as to whether the two are intimately related.

The livers from infected chick embryos were homogenized, washed free of cytoplasmic material, and extraction of nucleoprotein was done in molar NaCl solution. Preliminary tests demonstrated that the virus remained active in solutions of this strength. Titrations of virus in mice, and DNA determinations were carried out at different stages upon the fluid and sedimented parts of the preparations. The supernatant fluids were removed from suspensions centrifuged at 10,000 rpm for one hour. The sediments were then ground and the supernatant tested after centrifugation at 8000 rpm for ten minutes. Six experiments of this nature were done with virus concentrations varying from LD50's of 10^{-4} to 10^{-6} and the results of two typifying the procedure are presented in Table 3.

TABLE 4

ADDITIONAL DETERMINATIONS OF DNP CONTENT AND VIRAL ACTIVITY

	M/1 NaCl extract				*DNP 1x ppt. + diss.*	
	Crude DNP		*Sediment*			
Experiment	*Virus*	*DNA*	*Virus*	*DNA*	*Virus*	*DNA*
12	>2500	222	4000	72	100	156
13	5600	192	4000	34	750	182
16	25,300	261	20,100	18	2700	231
23	39,000	199	*1350	13	2512	177

* Centrifuged at 10,000 rpm 1 hour instead of 8000 rpm 10 minutes.

The data herein presented show a definite dissociation between the DNP content of the tissue or its extracts and the amount of virus detectable in them. The first washing constantly had a high titer of virus and a low DNA content. The extracted nucleoprotein showed a reduced titer of virus although the DNA determinations were consistently high; moreover, the virus titer of the tissue from which the DNP had been largely extracted was greater than that of the fluid DNP.

From four earlier experiments the data in Table 4 were obtained. They emphasize the fact that there is a disproportion between the DNA content

of material extracted from infected tissue, presumably as DNP, and the viral activity; that the DNA remained largely in the soluble extract, whereas the sediment possessed about the same amount of infectious virus with a much lower content of DNA.

Discussion

These investigations were undertaken in the expectation that evidence could be gained as to the localization of herpes virus in the cell which it infects. The development of nuclear inclusions in the infected tissues has resulted in a widespread opinion that the inclusion represents a selective localization of the virus. And it was this concept which suggested the present studies. Contrary to expectations, however, the evidence obtained indicates that herpes virus is not primarily associated with the cell's nucleus. Under the conditions of experiment the data showed:

1. That herpes virus did not selectively combine with washed nuclei of apparently susceptible cells;
2. That herpes virus was not found in greater concentration in the nuclei of infected cells than in preparations containing primarily mitochondria or microsomes;
3. That the nucleoprotein extracted from the nuclei of infected cells is not proportionately related to the amount of active virus demonstrable. In fact, purification of the nucleoprotein by simply precipitating and redissolving it results in a great diminution in virus titer while the yield of nucleoprotein is changed but little.

It has been repeatedly noted that herpes virus is readily released from infected cells. The observations of the present study are in keeping with that experience since it was noted that washing of infected tissue which removes the great bulk of cytoplasmic material, including RNP, also takes out a high proportion of active virus. Moreover, since this washing does not seriously affect the nuclear content of DNP, the results suggest that the virus is more richly associated with cytoplasmic material.

Certain obvious questions concerning interpretations can be raised. First, is the failure of the separated nuclei to combine with virus merely a reflection of altered physiological state incident to the process of preparation? In this respect one can state that they appear relatively unchanged in staining characteristics. Furthermore, most work in which specific cell-virus union has been demonstrated *in vitro* has been done with material which has been subjected to washing and which is not in its normal physiologic environment. A second question relates to the possibility that the infected nuclei are broken up so that the material most likely associated with virus

is washed away. The nuclei remaining or the nucleoprotein extracted would represent, in this case, the unaffected cells of the tissue. The amount of DNP obtained, however, from the infected embryonic liver is essentially the same as that derived by the same procedures from normal liver, and the number of nuclei obtained from normal and infected liver is approximately the same.[1] Furthermore, sections reveal a high proportion of affected cells so that in some areas nearly all the nuclei contain inclusions. A third question is whether the quantity of DNP which might be virus is so small that it would of necessity be but a small fraction of the extracted nucleoprotein. This is expected to be the case but even so, some parallel between the total amount of DNP and the virus titer should be expected. And if the virus were composed primarily of DNP of nuclear origin, the two should accompany one another quantitatively. The results, however, lead to the conclusion that the virus activity is not related to the DNP but to material of other nature. This is in accord with the interpretation of Cowdry who pointed out that the inclusions of herpes did not show Feulgen reaction.[6] They indicate, too, that the nuclear inclusions are not virus itself but are changes perhaps secondary to virus injury at the cell surface or in the cytoplasm.

Conclusions

Although the inclusions produced by herpes virus are nuclear in location, no evidence was obtained to indicate that herpes virus is selectively bound to nuclei or to nuclear nucleoprotein. The results indicate that the inclusions are not herpes virus.

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SOME NOTES ON THE YALE UNIVERSITY SCHOOL OF MEDICINE WITH SPECIAL REFERENCE TO MILTON WINTERNITZ

JOHN F. FULTON

I am particularly happy for this opportunity to express the warm appreciation which all feel who know what Milton Winternitz has contributed to the Yale School of Medicine and to the advancement of American medical education. Many have derived inspiration from his example, but for the most part it is unrecorded. Various aspects of the growth of the School come to mind as we turn memory back to the time when Dr. Winternitz, or Winter, as we now call him affectionately, first came to New Haven. How much of the "blood, sweat, and tears" that went into the first years of rebuilding the School along the lines of Winter's vision will find their way into this volume, it is hard to say. But one knows that those were stirring years, filled with battles, hopes and disappointments, and phenomenal progress. I regret that I arrived too late to witness the thrilling metamorphosis which took place during the 1920's, for it was not until much had already been accomplished that the opportunity to join the Yale faculty came to me.

Since many doubtless will draw—appropriately—on personal reminiscences on this occasion, I turned to my diary to refresh my memory on the details of my first visit to the Yale School of Medicine. There appeared in the *New York Times* of April 6th (1950) a column on diaries and diarists. It was sent to me by a friend who knew that I had begun the practice, often tedious, as a means of keeping a widely scattered family supplied with the details in which families are interested. The *Times* editorial concluded that "perhaps the best diarist is he who keeps a diary out of curiosity, for the pleasure of removing occasionally from a dusty shelf his record of some past year and of reading there what he wrote when he was younger; how wise was he then, or how foolish, and what beliefs tenaciously seized on then have stood the tests of time and experience?" In regard to the present excerpts, I shall have to let the reader answer these questions for himself.

> I left [Boston] on the 8:30 train and arrived at Sterling Hall, New Haven, at 12:30 [on February 8, 1928]. Dean Winternitz is a very attractive and young pathologist who has great dreams and ideals for the Yale Medical School. He talked to me for the first half hour concerning

the aims and future of the School, emphasizing particularly their desire to secure greater academic freedom by doing away with hard and fast academic prerequisites and regulations. They appear to be following more the English system of letting a man do pretty much what he likes during the first two years of his medical course and at the end of that period having an examination to see how much he has taken in. All this seems very attractive. They aim furthermore to reduce didactic lectures to a minimum in order to give the students (and professors) greater opportunities for cultivating their own interests.

Their plan for the future for their department of physiology is rather a peculiar one. At the present time Professor Lafayette Mendel of the Department of Physiological Chemistry is the acting chairman of the Department of Physiology. In addition three separate posts are to be filled by individuals who are to be termed Physiologists. The first one is an assistant-professorship devoted to physical chemistry. The second is also an assistant-professorship now devoted to metabolism and blood gas analysis. A third position, an assistant or associate-professorship, is to be given to one interested in the more general aspects of physiology and they are attempting to secure someone experienced in the physiology of the nervous system in particular. Each one of these posts has separate budgets and salaries. . . .

After the Dean had outlined these things he took me to lunch with Professor Raymond Hussey, Associate Professor of Pathology, at the Graduates Club where we were joined a little later by Professor Harold S. Burr, a rotund and amusing young neuroanatomist at Yale. They were all very agreeable.

After lunch Dr. Hussey piloted me around from one department to another to meet the various people in the Medical School. . . . At 4:30 they all came to a tea which was given in the Dean's Office, apparently in my honor, which seemed to be a rather awkward occasion for them for no one seemed to know just what advances had already been made to me. After the tea I had a further talk with Dr. Winternitz and then to Professor Hussey's house for cocktails after which I was taken to a dinner of a convivial medical organization known as "The Ladies' Aid Society," so named because it was founded by a cheerful group of obstetricians. I sat at the dinner beside Dr. Sam Harvey, the neurosurgeon of Yale, a quiet and very amusing individual. The speaker of the evening was a Dutch Reformed minister. . . .

Since there were doubts and uncertainties on both sides and since I was keenly conscious of my lack of training, I decided to decline Dean Winternitz' attractive offer and return to England for a time where I had been offered a teaching fellowship in physiology at Magdalen, my old College. It also gave me further opportunity for association with my former chief, Sir Charles Sherrington. A little over a year later Dr. Winternitz turned up in Oxford and as a result of his visit, I made a second

trip to New Haven—in August of 1929—which was also duly recorded. On the first day Dr. Harvey hospitably entertained me and took me about the University. On the second:

> After discovery of where the general postoffice is, I went directly to the hospital and saw Dr. Harvey's clinic and an operation upon a case of Jacksonian epilepsy which proved to have a granuloma with localized arachnoiditis. The bone-flap was turned down by his assistant, Bill German, a friend of mine from the Brigham, and everything went very smoothly.
>
> Later in the morning I had a long conference with Dean Winternitz about the financial arrangements and plans for the Department. He is an astonishing man; he has made the School what it is—first the Sterling Hall of Medicine, then the New Haven Hospital, later the departments of surgery and pathology, now the Institute for the Study of Human Relations, for which ground is just being broken. Singlehanded he has planned, financed, and had constructed this truly remarkable group of buildings, all in ten years. Along with this gargantuan undertaking, he has taught pathology, developed an elaborate museum and built up and developed a superb system of record-keeping which really outdoes that of the Brigham. Finally he appears to have filled the School with good men. He is of Napoleonic temperament and build, and a thoroughgoing autocrat but honest as the day is long and possessed of a broad and sympathetic nature.

Dr. Winternitz came to the School at a low point in its existence. The School of Medicine—or the Medical Institution of Yale College, as it was originally called—had been fortunate in its founders. When in 1813 thirty-seven students enrolled in the new school, the president was Timothy Dwight, the elder, who had had the vision to see the need for a school of medicine in Connecticut; and the medical faculty which had been assembled included the eminent New England physician and founder of the medical school at Dartmouth College, Nathan Smith, as Professor of the Theory and Practice of Physic, Surgery and Obstetricks; Eneas Munson and Eli Ives, New Haven physicians of excellent reputation, as Professor of Materia Medica and Botany and Adjunct Professor of Materia Medica and Botany respectively; Benjamin Silliman as Professor of Chemistry and Pharmacy; and Jonathan Knight, as Professor of Anatomy. The new school flourished because there was urgent need for medical education, Yale being only the sixth school to be founded in the United States. (Philadelphia was the earliest, followed by Kings College [later Columbia], Harvard, Dartmouth, and Maryland.) Its reputation remained high throughout much of the nineteenth century, but as time went on, the School began to flounder despite the efforts of a series of substantial clinical

teachers who transmitted a knowledge of the Art and stimulated successive generations of students. Much of the difficulty during these years was lack of funds, for the School needed laboratory and library facilities and much else. But above all, it needed inspired leaders with scientific imagination.

All this was recognized by one of Yale's distinguished alumni in medicine, William H. Welch, when he was asked to speak in 1901 at the two hundredth anniversary of the founding of the College on "The Relation of Yale to Medicine." He wrote that it "is about the most barren theme which I ever tackled. The relation is so slight that I shall have to beat around the bush and talk on side issues. If they had only asked me to talk on the Relation of Yale to Calvinism or Football there would have been something to say."* In spite of this note of discouragement, uncharacteristic of Welch, his speech bore no shadow of it. He reviewed Colonial medicine in the eighteenth century with mention of the part played by graduates of Yale who had become physicians and then turned to the founding of the Medical Institution and gave an excellent review of its history. But as he approached the end of the century, there was less to say, so he looked toward the future in his concluding paragraphs:

> In centuries past the greatest renown of many universities lay in their medical faculties. There have been later times when the conditions of medicine and of medical education made it less fit to enter into the life and ideals of a university. It is not so to-day. Medicine has now become one of the great departments of biological science with problems and aims worthy of the highest endeavor of any university, surely none the less worthy because they are associated with human interests of the highest importance.
>
> The union of medical school and university should be of mutual benefit. Medicine needs the influences of a university for its highest development, and the usefulness and frame of a university are greatly increased by a strong medical department. There is today no direction of scientific research more productive in results of benefit to mankind and in the increase of useful knowledge than that upon which medicine in these latter years has entered, and there can be no nobler work for a university than the promotion of these studies.
>
> But medical teaching and research can no longer be successfully carried on with the meagre appliances of the past. They require large endowments, many well equipped and properly supported laboratories, and a body of well paid teachers thoroughly trained in their special departments. With an ampler supply of such opportunities as these there is every reason to believe that the Yale Medical Department would take

* Flexner, Simon and Flexner, J. T.: *William Henry Welch and the heroic age of American medicine.* New York, The Viking Press, 1941, p. 58.

that important position in the great forward movement of modern medicine to which its origin, its honorable history, and the fame of this ancient University entitle it. May the next Jubilee find medicine holding this high position in Yale University!*

In 1917 Winternitz, one of Welch's students, came to Yale fired with enthusiasm for the Johns Hopkins experiment in medical education under which students were given wide elective opportunity and under which, also, they had access in their third and fourth years to the wards on their own time. Groundwork for the revolution that was shortly to take place had been well laid by George Blumer, another Hopkins man. But Dr. Blumer had less of a fighting spirit, and to pull the School into the forefront of medical institutions in the United States required the kind of dynamic energy which Winter had in the fullest measure. To some of the more conservative, his ideas were revolutionary, for hospitals in their eyes were intended merely to cure patients and not to teach students. But within a few years of his advent at Yale, the School of Medicine had become a modern and forward-looking institution and had assumed once more the high position it had once held among the medical schools of this country.

Fortunately, at the time when Winter was attempting to modernize the School a man of rare understanding and imagination became president of Yale. James Rowland Angell assumed office in 1921 when Winter was having something of a crisis with his faculty. Diplomacy in human relations was not always one of his fortes, but with Mr. Angell behind him—and Mr. Angell believed in him thoroughly—many of the more difficult problems facing the School were effectively solved.

As the present chairman of the Medical Library Committee, I should like to outline briefly one of Winter's principal contributions to the School to which little public reference has been made—the foundation of the Yale Medical Library. I am reminded of the story of that colorful Viking, Olof Rudbeck who, when the town of Uppsala burned in 1702, stood on the roof of the University library, his white hair illumined by the flames, directing the firefighters below in stentorian tones (which our Winter is also capable of using). Although informed that his own house and the work of fifteen years on a great botanical treatise were burning, he stuck to his post for he knew that if they could save the library, there would still be a university. From his experience at the Hopkins, Winter also knew that if we were to have a good medical school, we must first have a good library, for this would always serve as the primary laboratory of instruction.

* Welch, W. H.: The relation of Yale to medicine. Yale M. J., 1901, *8*, 127.

Accordingly, the dust was removed from a collection of medical books given by Dr. T. C. Janeway, Yale '92 (which had been stored for three years for lack of space), and a library was established in one room of the newly completed Brady Memorial Laboratory. To this nucleus, books and journals were added through the generosity of George Blumer, Frank P. Underhill, H. B. Ferris, Winter himself, and other members of the staff; and when the first librarian, Miss Laura F. Philbrook, made her initial report on June 30, 1918 there were 1,500 bound books and periodicals, 100 volumes of unbound periodicals, and 19 pamphlet cases full of reprints and duplicates.

Winter continued to foster the growth of the library from its beginning in Brady and through its years in larger quarters after the Sterling Hall of Medicine was built. When he retired from the deanship in 1935, after fifteen years at the helm, his last official request to the Yale Corporation was for funds for the drawing of plans for a new medical library—plans which were to culminate in the present Y-shaped library wing, completed five years later.

Writing from Paris after two months' study of the French journals and informal association with French colleagues, I have come to appreciate in what high regard our School is held abroad. Some have declared French medicine chauvinistic, but it has been my experience that they are far better informed concerning our literature than we are concerning theirs, and they set an example for international co-operation which we might do well to emulate. Winter's vision of a medical school was international in scope, and I believe that it is being effectively realized.

I have always enjoyed pointing out to various members of the Hopkins faculty that the Johns Hopkins is really an offshoot of Yale since Daniel Coit Gilman, the first president of Hopkins, received his training in New Haven, and two members of the first and famous faculty, Welch and Halsted, were graduates of Yale College. But I feel that the Hopkins discharged the very considerable debt it owed to Yale when, in return, it sent us Milton Winternitz.

VARIOUS ASPECTS OF ADOLESCENCE*

J. ROSWELL GALLAGHER

Those few physicians who confine their attention to adolescents are not infrequently asked in what manner and to what degree the health problems of that age group differ from those of a child or from those of an adult. Adolescents do present problems peculiar to their age, but they are not grave ones and they do not require the creation of another specialty. The proper care of adolescents requires only that the family physician, the pediatrician, parents, and teachers treat these young people as adolescents, not as little children, not as adults; they are not little boys or little girls any more, and they are not adults yet: they are young people who are beginning to be adults. When this simple fact is really understood, many of the matters peculiar to adolescence will no longer appear to be problems; until it is, the adolescent will continue to seem to many to be an enigma.

If any one specialty were to take over the care of adolescents, it should be pediatrics. The problems of growth, the contagious diseases, the behavior problems, and the blood and bone disorders which the pediatrician cares for in childhood should be observed and treated by him until his patient becomes an adult. Too often such a patient is abandoned just because he is fourteen: that would seem an inadequate reason for changing from the doctor who for many years had cared for the results of poliomyelitis, retarded growth, friction with parents, or asthma. Those, by and large, are not problems of adulthood—they will have been straightened out by the time the growth process is over. Until that time it would seem best for the patient to be retained by the physician who cared for those ailments from their beginning.

There is no better way both to explain and to reiterate the necessity of interpreting and handling these young people in terms of the age group to which they belong than to discuss a few of the conditions which are not only common in them but which also are of particular significance *to* them. The limitations of this writer's experience is the only excuse for confining these remarks to the male adolescent; the female at this age is just as worthy of special consideration.

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* From the Department of Health, Phillips Academy, Andover, Massachusetts. Studies upon which this paper is based were aided by a grant from The Grant Foundation, Inc.

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Growth and development

A study of the growth and development phenomena of adolescents furnishes an excellent means of understanding the problems peculiar to them. These are matters which deeply concern them. The small child or the thirty-year-old adult cares little about his height or state of maturity, but the fifteen-year-old wants to know how tall he will be, when he will thin out, and when his beard will appear.

Parents and teachers as well as the adolescent himself need to know something about growth in this age group. They find it difficult to understand that there is nothing orderly about growth, that there is no one best way of growing, that there is neither an ideal type of behavior nor an ideal size at any given age. The boy who is tall and heavy and bearded at fourteen is expected to "act like a man" and yet if he asks for the privileges which his slight and beardless seventeen-year-old brother has been granted, he is firmly reminded of his age. All this confusion can be avoided if one will regard chronological age with some suspicion during the adolescent years. These young people vary too much in the rate and manner in which they grow and mature to make chronological age a good yardstick.[19]

The evaluation of the hand-wrist x-ray provides a skeletal age[18] which is a more suitable index of the adolescent's growth and maturity status than is the figure based upon his birth date. Hand-wrist x-rays of two boys both fourteen years and eleven months of age are shown in Figure 1. In contrast to their similar chronological ages, their skeletal ages are forty months apart. These were both normal healthy boys. They differed in a natural way in their rate of growth, but there is no reason to suspect that each was not growing properly, that one will not mature, or that the other has grown too fast.

The variation in state of maturity which one finds at any given age during adolescence is also illustrated in Figure 2. Degree of maturity,[11] based on the status of secondary sex characteristics, can be rated objectively and will be found to parallel closely the skeletal age ratings. The six boys in Figure 2 were all fourteen years and nine months of age. They have been arranged in order of ascending skeletal age (based on their hand-wrist x-rays): these skeletal ages ranged from thirteen years two months (nineteen months below his chronological age) to seventeen years three months (thirty months above his chronological age.) Their maturation ratings[11] ranged from three to five.

The facts which parents and teachers and the boys themselves need thoroughly to understand is that among perfectly normal adolescents there

is great variation in size and in state of maturity, that there is no one way of growing which is better than another, that true abnormalities in growth are extremely rare.[5] To expect the boy at the left in Figure 2 to behave in the same manner as the boy at the right, to treat him in the same manner, to hold him to the same duties, studies, and responsibilities is neither entirely logical nor always successful.

That real abnormalities are rare, that almost every child will eventually grow and mature is hard for both parent and child to understand: they desire that what they consider a standard average pattern be followed. It will help to explain patiently that each will, and is entitled to, grow in his own way. That these ways differ, but that as long as there is no true abnormality and as long as there is some evidence of progress no matter how slight, all will be well, is shown in Figures 3a and 3b. Figure 3a shows three boys whose chronological ages were fourteen years seven months, fifteen years five months, and fourteen years nine months, whose maturation ratings were 3, 4-5 and 5, and whose skeletal ages were thirteen years six months, fifteen years three months, and fifteen years eight months. In Figure 3b these same boys are shown eight years later; at that time there obviously was no appreciable difference in their maturity, masculinity, or size.

Infectious diseases

In connection with infectious diseases during adolescence it is important to remember that members of this age group may have lost the benefits of the immunizations which were given them in early childhood. It is common practice nowadays to vaccinate against smallpox and to give toxoids to immunize against tetanus and diphtheria during the first year. Adolescents are, in general, a healthy group: many of them rarely go to a doctor and it is easy to overlook the renewal of their immunity against these particular diseases. The adolescent who has not been vaccinated or who has not been immunized against diptheria[18] or tetanus for several years certainly requires that these procedures be repeated.

The tendency to protect young children from mumps, measles, chickenpox, and German measles is ill-advised. Mothers, overly apprehensive concerning all illness, and teachers too conscious of school attendance records, often try to avoid exposing children to these diseases. It is never convenient to have mumps or German measles,[6] but it is better preventive medicine to allow children to have these and other contagious diseases during their early years when these ailments are often less severe and of less consequence. There is also much to be said for encouraging parents and teachers to accept these minor illnesses as a necessary evil, more appropriate to childhood

than to later life, and definitely not as a proper source of anxiety. Rumors about an epidemic, a frantic searching for and isolation of contacts the moment a case of chickenpox appears in the fourth grade is poor health education and worse mental hygiene. The only proper method of controlling these illnesses among healthy children in adolescence (except for the use of gamma globulin for measles modification[15]) is to urge that they have them while they are still little children.

Health education

Physicians are not infrequently asked to serve upon school boards and may well be asked what constitutes proper subject matter for a health education course. It seems fair to say that the attitude which a boy has toward his health is the most important part of his health education. The avoidance of anxiety toward illness and the avoidance of the pill habit should start in infancy but they will need reinforcement as the boy gets older and is subjected to fear advertising, his playmates' habits and attitudes, and the peculiar ways of some of those adults whom he learns to admire. If the adolescent has not developed a rational, objective attitude toward his health, it is not too late to try to give him one. To interest him in finding causes rather than taking pills, to help him to avoid worrying about his health and from being reckless is more important than that he acquire a smattering of physiology or learn the incubation period of measles.

Some factual information,[8] as well as a basically sound attitude, is important. The fat boy or the thin boy is usually willing to learn something about nutrition. Ways to avoid accidents are, for this age group, the most valuable of all health education subjects. Most of them want to know something about acne, whether strenuous exercise or smoking is harmful, how much sleep they should have. Sex education has real meaning for them.

A good many have questions about growth and development: a boy such as the one at the left in Figure 3a needs to be told a good deal about the way in which young people's growth varies one from the other. This boy, who was a little less than sixty-two inches tall and whose maturity rating was no more than three at his fourteenth birthday, needed reassurance and patient explanation. However, the fact that they ask only about their height should not lead one to believe that growth is their primary concern—they want to be tall, but it is their slowness to mature which deeply disturbs them.

Medical findings

The proper medical examination of members of this age group requires that one know something of an adolescent's attitude toward health and that

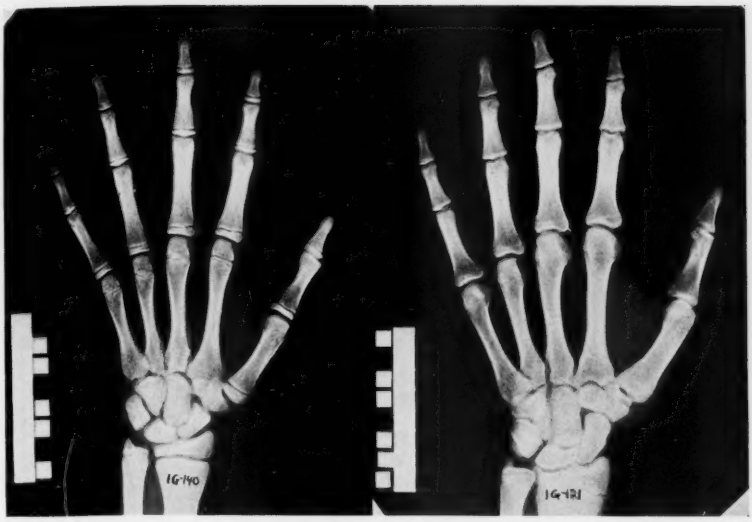

FIG. 1. Hand-wrist x-rays of two boys, each of whose chronological age was 14 years 11 months. Evaluation of these films yielded a skeletal age rating of 13 years 6 months for the x-ray at the left (IG-140) and of 16 years and 10 months for that at the right (IG-121).

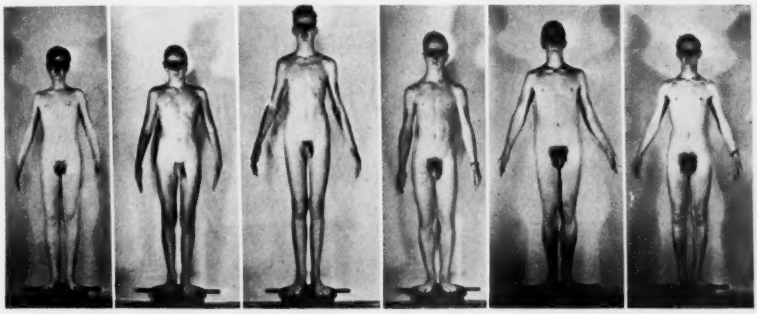

FIG. 2. Each of these six boys was photographed when his chronological age was 14 years 9 months. Their skeletal ages, however, were (from left to right) 13 years 2 months, 14 years 1 month, 14 years 9 months, 15 years 7 months, 15 years 11 months, and 17 years 3 months. Their maturation ratings were 3, 3-4, 4, 4-5, 5, and 5.

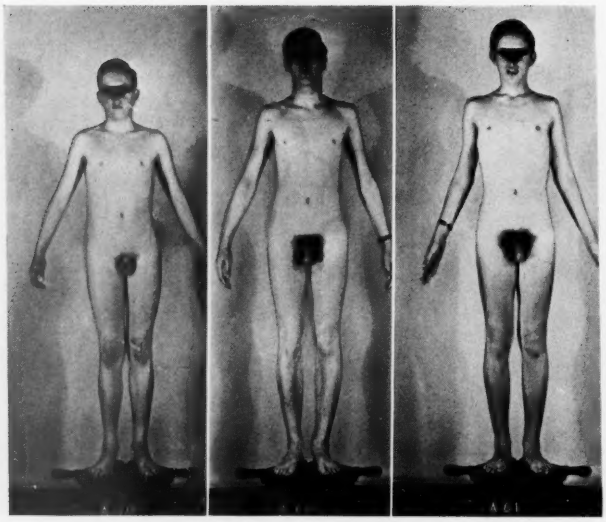

FIG. 3A. Three boys photographed when their chronological ages were 14 years 7 months, 15 years 5 months, and 14 years 9 months. The skeletal ages at that time were 13 years 6 months, 15 years 3 months, and 15 years 8 months; and their maturation ratings were 3, 4-5, and 5.

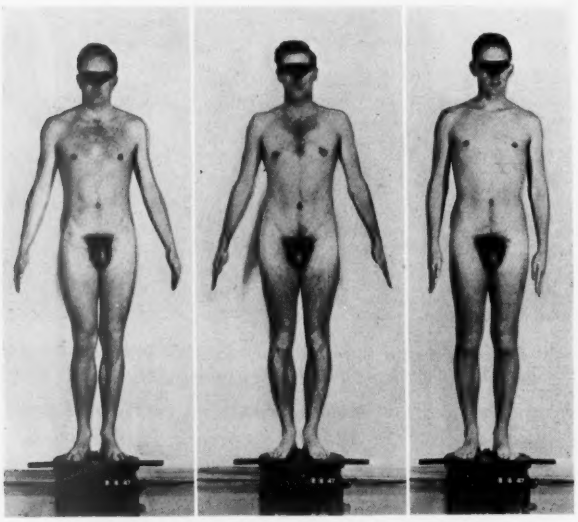

FIG. 3B. The boys shown in Figure 3a were again photographed eight years later. The rates at which these three boys matured were very different, but the end results were similar.

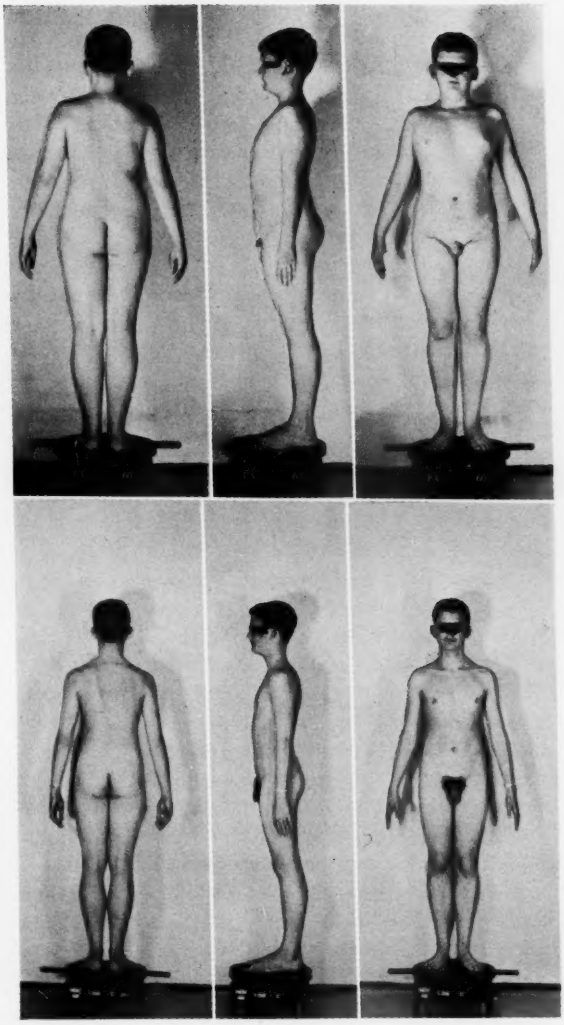

FIG. 4. The upper photographs show a boy at 13 years 8 months and the lower ones show the same boy two years later. No therapy was used.

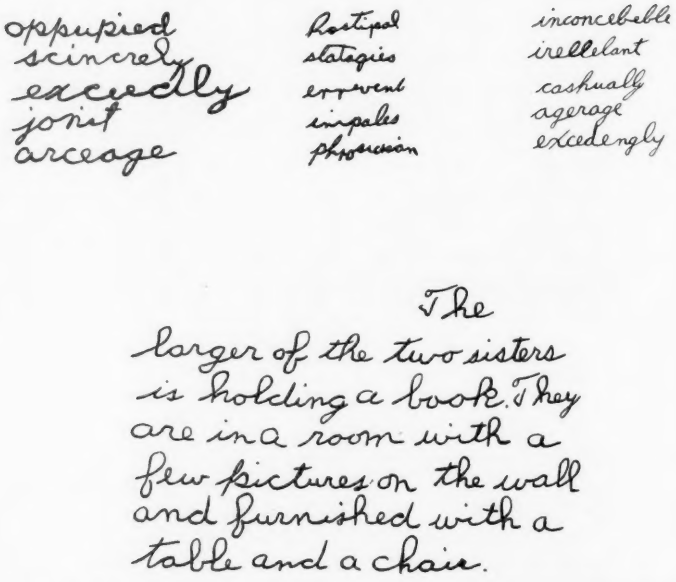

FIG. 5. Reproduction of parts of a written spelling test given three boys who had a specific language disability. The excerpt from a theme was written at the same time by the boy whose spelling is shown at the upper left.

some of the findings be interpreted in terms of the patient's age. Adolescents tend to deny that there is anything wrong with their health; they resent the implication that there is anything abnormal about them. The adolescent wants to be like everybody else: in his presence it is well to avoid careless comments about something "interesting" or about some "abnormality" which has been found. Most important to remember with this age group is that it is now time that the physician become the boy's doctor, that he go to his physician alone and with his problems. No longer is he a little boy being brought by his mother with her problems.

There are not only special things to look for but there are also certain findings apparently to overlook when one examines these young people.[7] Systolic hypertension is common, particularly in those who are not used to going to a doctor but it is only very rarely that it has an organic basis. It may, however, be of real significance: a fair proportion of those adolescents who have considerable systolic hypertension at a medical examination may be the tense, anxious, or even hypertensive adults of tomorrow, and they would do well to learn to live in a more relaxed fashion. Albuminuria is fairly common, but it is usually of the orthostatic type. Its most important aspect is the frequent need to reassure both parent and boy that its presence does not indicate the existence of serious renal disease.

A frequently misinterpreted finding in adolescence is the basal metabolic rate. If the mother is being given thyroid extract or if the boy is overweight or is doing poorly in school, a mother may suggest that the boy have a metabolism test. The chances of the metabolic rate being somewhere between minus ten and minus thirty are far from remote, but it is most unlikely that to give him thyroid extract will be necessary. Even if his thyroid is, at that particular time, apparently less active than normal, there is no reason to believe that it will not soon adequately adjust itself. In rare instances when a boy's skin is very dry, his behavior sluggish, his heart rate and metabolic rate persistently low, and his serum cholesterol high over a considerable period of time, the use of thyroid extract in doses adequate to constitute a therapeutic test is justifiable. In the usual case, however, it is much sounder medicine and infinitely better mental hygiene patiently to explain how frequently normal variations in growth pattern and thyroid behavior occur.[3, 10]

What thyroid extract can accomplish for those rare patients who need it is well known. Figure 4 shows what *time* can do. At age thirteen years and eight months this young boy weighed 127 pounds and was 61.6 inches tall. Two years later, at the time the lower photographs were taken, his weight was 132 pounds and his height 67 inches.

At an adolescent's medical examination there are a few matters to consider which are of more importance to him than to younger or older people. Acne is the bane of his existence.[2] It is just as important to try to treat it as it is to explain its relationship to the maturation process. Status of color vision is of importance to the boy who plans a career in the army, navy, air corps, or merchant marine. A pilonidal sinus excludes many a boy from the NROTC program. Plantar warts interfere with his athletics. A testis still undescended after puberty has begun will probably require an operation. Not of least importance at the adolescent's medical examination is his chance to ask questions, to talk over things which may have bothered him. It is better to give him a chance to talk, an opportunity to ask questions, than it is to make suggestions: the relationship the physician builds up with him may do more for him than much of the advice the physician can give.

Low back pain is a symptom which is all too common in adults and which unfortunately is not unusual in adolescents. In this age group whenever there is persistent low back pain, it is imperative that X-rays be taken to determine whether or not such a disorder as epiphysitis or spondylolisthesis is present. Strapping with adhesive, heat, sleeping on a bed board all help, at least during the acute stage, but in order to prevent recurrences it is well to consider the advisability of so strengthening the erector spinae muscles that they may subsequently be capable of carrying a greater proportion of the task of supporting the back.

Progressive resistance exercise has been found effective in strengthening the hyperextensors of the trunk. A small group of patients[8] who have been given this type of exercise (but only after careful clinical and X-ray examination) have apparently been considerably benefited.

Emotional and personality disorders

Severe psychiatric disorders are not common in adolescence, but the number of these young people who have minor disturbances of their mental health is large.[14] The more serious demands and problems which face them in later life—marriage, getting a job—are not yet theirs, but failure in school, parents' divorce, death of a friend, failure to mature or grow, rebellion, excessive dependence, antagonistic feelings toward either parent, lack of friends—any of these can act as a precipitating factor in the development of a disorder which should have attention. Each will react to such factors in a different way. To change from school to school will mean nothing to one boy and may thoroughly upset another who is more dependent or who needs the support of friends about him. A harsh, critical

father may bother one boy very little, another will be in a turmoil of rebellion against all for which that father stands.

Adolescents' mental disorders have two characteristics in which they differ from an adult's: their disorders often appear to be of much worse prognosis than subsequently turns out to be the case, and they usually talk more freely and respond more rapidly to treatment than do adults. The term schizoid is often used too loosely in describing an adolescent's behavior. An adolescent may be very confused and disturbed and yet make a rapid recovery; his symptoms may be similar to those of an adult who really is schizoid, but the course of his illness is so frequently different from the adult's that the term's implications make its casual use ill-advised. This is not to say that all these young people need is a friend or a patient listener: frequently they need much more than that. However, they do seem to benefit most when they are not regarded in too pessimistic a fashion and when their symptoms are thought of in terms of their years and not as an adult's would be.

School failure

Failure in school is of so much importance to an adolescent that it often has to be considered in relation to some of his physical and psychological difficulties. Nausea, headache, fatigue, behavior problems—all those and many more can and do arise out of failure in school. Rapid growth, low metabolic rate, anemia, eye strain, and fatigue are too frequently blamed by the parent for the child's failure, and their emotional disorders too often overlooked.

The causes of failure in school are many.[4] It is just as desirable that headache be recognized as a possible symptom of failure in school as it is that anemia not be uncritically accepted as the cause of low marks. An objective approach with an orderly, thorough investigation of the possible physical causes, then an evaluation of such factors as intelligence, proper grade placement, conflicting interests, the presence of a specific language disability, and finally an investigation of personality and emotional factors is essential. Lack of friends, rebellion against a parent, disinterest in the type of studies, confusion because of a friend's death or his parents' divorce may so affect a boy as to make him appear stupid, lazy, or indifferent to the teacher. Not to be forgotten is the "slow starter," the boy who for some unknown reason plods along in a mediocre way for years, gradually improves, and finally confounds the wisdom of teachers and the validity of tests by becoming an outstanding scientist or lawyer.

One cause of scholastic failure, the specific language disability, is worth

special consideration.[4, 9, 14] Between five and ten per cent of boys find it difficult to read; they spell in a bizarre fashion, have cramped, uneven handwriting, may have some speech disorder, and may give a history of similar handicaps in other members of their family. A considerable number of these boys are ambidextrous or have evidence of a mixture of cerebral dominance such as right handedness and left eyedness. As they grow older and their difficulties in school increase they may show the effects of discouragement and frustration. Early recognition of this condition can prevent a good deal of trouble. It is best that these children defer for a year or two—until they are ready—the time they start to read and spell, that every effort be made to develop their visual, auditory, and kinaesthetic imagery for symbols, that they be given drill in phonetics, that they *not* be taught to read by the "flash card" method.

Some, whose disability is not severe, and whose intelligence is high, are able to get through the early grades but begin to fail when faced with the more difficult work of high school. In Figure 5 are reproduced the handwriting and spelling of three fifteen-year-old boys of high intelligence. The uncertainty of their handwriting is shown in the variation in slant, the differences in pressure, the retracing. Reversals of letters, omission of syllables, and the confusion of one sound and letter for another are obvious. These three boys were also poor silent and oral readers and in the family of each another member handled words poorly.

The brief excerpt from a theme at the bottom of Figure 5 is included to illustrate the point that these cases may go undetected if one inspects only writing in which the boy had a free choice of words. That excerpt is from a theme written on the same day by the same boy whose spelling is shown at the upper left in Figure 5. He had learned to write without error within his own small vocabulary.

Patient, meticulous drill on phonetics, the building of strong association patterns between the sound, the appearance, and the feeling (in writing) of letters produced within a year vast improvement in those boys' fundamental skills. The teaching techniques used are undoubtedly of primary importance, but the psychological effect of having someone recognize and help such boys with the source of their frustration and failure must be of considerable importance.

An inspection of a spelling test is the best single means of detecting this type of trouble: it is the kind, not the number, of errors which suggest the diagnosis. It is important to distinguish these children from that much larger group who through lack of interest, low intelligence, poor teaching,

or some emotional or personality disorder, read poorly. Many of those children may respond very well to various methods designed solely to increase their reading efficiency either by direct attack upon it or by attempts to motivate them or by efforts to resolve their emotional conflicts.

Eye glasses, vitamins, more rest, iron, tonics, thyroid extract, or more discipline will not solve the problems of the boy who fails in school because of lack of interest, low intelligence, a language disability, or the confusion and anxiety growing out of a personality or emotional disorder. Anemia, rapid growth, metabolic disorders, and poor vision or hearing all are important, but only rarely is a single handicap as significant as is the nature of the boy's personality, aptitudes, and adjustment.

Athletics

Adolescents take games seriously. They are important to them as an outlet for their excessive energy, as exercise, as an opportunity to learn cooperation, and as a means of learning to win and lose gracefully. Not to appreciate their significance to a boy, casually to suggest that a boy give up a game is to make a considerable error.

There are very few adolescents who should not be allowed to compete in athletics. The danger of their being strained if they are properly conditioned,[10] or of their being injured if they are adequately protected,[17] should not be a deterrent. A boy who has heart disease can play in the goal at soccer, hockey, or lacrosse: it is more desirable to try to make him feel he can be part of a group than it is to overprotect him. More exercise rather than more rest is the proper prescription for the boy who tires too easily because he is in poor condition: the more he takes it easy, the more fatiguing will subsequent exercise become—too much restriction, too much solicitude, too much rest not only make a boy vulnerable to fatigue but also start him on the way to becoming self-centered and neurotic.

Knee injuries are so common and often so serious in adolescents that they deserve some special consideration. The management of the knee sprain during its acute stage needs little comment, but in its care during convalescence a few points which have general application are sometimes forgotten.[8] An adolescent's knee will not be strong enough so that he should return to athletics until it has become as strong as his uninjured leg. For the knee to have regained full extension and flexion, to be free of pain and fluid, and to strongly resist the examiner's hand is not enough. Ideally the quadriceps strength should be increased so that it has become stronger than it was when the injury occurred. Progressive resistance exercise[8] is a technique which during the past few years has proved to be a very useful

adjunct in the physical therapy of knee injuries. An adolescent's response to this type of treatment is much more enthusiastic than is his reaction to the suggestion that he wait for nature to heal him.

These are a few of the matters which are of importance to adolescents. There are few baffling problems if one learns a little about their attitudes, behavior, needs, and physiology. They want—and need to be—regarded as people, not as problems. Their outstanding characteristic is their capacity for change: neither their attitudes nor behavior nor physiology has yet settled into their adult mold. That is the only reason for it being at times difficult to understand them and their ailments—it will continue to be particularly so if one attempts to judge them by adult standards.

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AN ATTEMPT TO INTERPRET A GREAT SCIENTIST'S CONTRIBUTION TO HUMANITY THROUGH MEDICINE AND NURSING

ANNIE W. GOODRICH

It is my great privilege and honor to attempt an interpretation of a great scientist's vision and achievements, a challenge far beyond my pen.

Milton C. Winternitz chose for his life work what I deem to be, if such comparisons are permissible, the greatest of professions. For to this profession the twentieth century has given a master key called Science, which is opening weekly, almost daily, the hitherto unknown, thereby blazing the trail for the *summum bonum* of man's long endeavor, "the one far off divine event toward which the whole creation moves," which may be interpreted as the insuring to coming generations that each member shall be well born. A true democracy implies the intrinsic value of every human being and demands that that human being be equipped with the good life, the essentials of which Bertrand Russell has epitomized as "intrinsic happiness, friendly feeling, love of beauty, and knowledge."

An even greater philosopher, John Dewey, has covered in ten words the essentials of a life worth living or, if you prefer, the pursuit of happiness—"The union of joyful thought with the control of nature." Never did genius more effectively build to this great end than has been exemplified in the life of Dean Winternitz.

It is a grave question whether the great medical center, its intent to extend its humane service throughout our beautiful state, would have come into being were it not for the vision of this great scientist, the dynamic current of whose will not only breathed life into a once sound foundation, but also raised the wherewithal through which only such great enterprises become realities.

It is difficult, indeed impossible, to interpret the full value of the unique building, designated as the Institute of Human Relations, a building of rare beauty that arose from one of the neglected areas of old New Haven. It was designed to bring into effective and integrated action the new sciences, psychiatry, psychology and sociology, and the modernized old sciences, biology, chemistry, and physics.

Dean Winternitz' vision resulted also in the creation of a gymnasium and recreation room in addition to study rooms and laboratories. With his

appreciation of the influence of beauty upon youth, the creation of the beautiful clubroom is not strange, its purpose to bring the young people of the Medical and Nursing Schools together in these artistic surroundings, where they were served by charming hostesses the beverage that stimulates but does not inebriate. Here they laid aside for a brief period each day their ardent tasks and in many cases forgot, for a short time, the somewhat dreary habitats in which some were housed.

No greater privilege could have been given to the nurse assigned the responsibility of the creation of the Yale University School of Nursing than to confer with the Dean of the Yale School of Medicine in the beautiful office which he had created, whose very walls whispered of the great thinkers of the past; for always his vision of things to be leaped beyond hers, and the door was always open to one who was not so well equipped.

No member of the little group that gathered together in New Haven in November, 1923, to outline a curriculum for one of the most momentous developments in the history of nursing education (the recently created Yale University School of Nursing), versed as they were in the difficulty of obtaining qualified instructors in the various subjects, could ever forget the reception given them by the brilliant members of the faculty of the Yale School of Medicine, who, despite heavy programs, offered to assist in any way the furtherance of the program of this school which this faculty of women expected would be looked upon as an unseemly intrusion into this great and revered men's university. As we consider the distinguished scholars, Dr. C.-E. A. Winslow, Dr. Arnold Gesell, and many other rarely gifted scientists gathered together by the most talented of Deans, Dr. Winternitz, we realize the undreamed-of strengthening of an already distinguished faculty of nursing specialists.

Whatever anxiety was felt by the small faculty of the School of Nursing was in no measure lessened by the fact that the Dean of the Medical School, and a number of his associates, had been moulded by the renowned Dr. Osler of the great Johns Hopkins, and by Popsy Welch, also of Johns Hopkins, and a native of Connecticut. It was, therefore, an evidence of wisdom that the Superintendent of Nurses, Effie J. Taylor, Associate Professor, was held in high esteem at Johns Hopkins; but there were others too—Elizabeth Melby, whose analytical mind solved the most difficult problems in nursing education, a balanced program of clinical experience, regardless of the demands of surgery and medicine over pediatrics and psychiatry; Helen Clarke who, as Registrar, gave valued assistance in the solution of our many problems of organization; Helen Stelling, instructor

in pediatric nursing; and several others worthy of mention, though not from Hopkins—Bertha Harmer, the eminent author of the *Principles and Practice of Nursing;* Amelia Grant, who organized the program of clinical experience in the outpatient department; Marguerite Robb (later Mrs. DuMortier), in charge of the operating room, highly qualified through her excellent surgical experience at the Peter Bent Brigham Hospital.

Never to be forgotten was one of the first conferences called in the former residence of the students of the Connecticut Training School with the ladies of the Connecticut School Committee and the Dean of the Medical School, a meeting which the Dean of the School of Nursing approached with timidity, praying to meet inevitable questions with tact. An opening remark of Dean Winternitz was, "Would you not think it advisable, Dean Goodrich, to have some form of hygienic shoes for the students to change to when they enter upon their clinical experience in the wards?" The ladies gasped! The Dean replied, "It is indeed, Dean Winternitz, an interesting suggestion. May I ask what form of shoes the interns change to when they enter the wards?" The ladies giggled. Dean Winternitz ejaculated, "You mean thing!" which affixed a seal of mutual understanding for all time.

Another memorable occasion was when the President called a meeting in his office to discuss the ever-rising costs of the two schools. Those present were Mr. Thomas Farnam, Treasurer of the University; Colonel Ullman, Chairman of the Executive Committee of the New Haven Hospital; and the two Deans. As the discussion waxed ever warmer, above the discord rose the powerful voice of Colonel Ullman: "But, Mr. President, you do not understand. We are dealing over there on that hill with prima donnas." Whereupon, the Dean of the Medical School arose and bowed profoundly to me. I responded, but, I fear, less impressively, being fully aware of the still existing inferiority complex of nurse to physician.

Was any school ever blessed with such an understanding President as Dr. James Rowland Angell? Each development presented its own problem. The question of office space for the School of Nursing faculty was easily met by Dean Winternitz' assignment of the third floor of Brady. A suitable residence for the students was a problem and necessitated a stroll with Dr. Angell and Dean Winternitz to inspect the possibilities, two of which were the "Dog House" and the former Elm City Hospital, whose beneficient service to the New Haven public had for reasons unknown been forced to cease. Keen eyes had already grasped how easily the latter structure could be turned into study halls and laboratories for the instruction, by the great teachers of the Medical School, of would-be young

surgeons and physicians. The briefest scrutiny of this building was convincing of its possibility as a residence for the students of the School of Nursing, if the required changes were made by an understanding reconstructionist. No words will ever express the gratitude to Mr. Frederick Johnson, upon whose already overburdened shoulders the heavy task fell. His remodelling of the gloomy halls and unattractive, well-worn passageways was almost unbelievable. Well-placed bookcases and architecturally beautiful fireplaces transformed the living room. No stone was left unturned to reconstruct Nathan Smith Hall, which has now started joyfully more than a thousand students on a life activity that, because of its worthwhileness, interprets in full measure the counsel of our forefathers as the life intent, "life, liberty, and the pursuit of happiness."

No students in the country were more privileged. It was interesting and exceedingly encouraging to have such authorities in science and medicine as Dean Winternitz, Dr. Lafayette Mendel, and Dr. Edwards Park urge as rapidly as possible the preparation of the teaching of the sciences by instructors qualified to determine the content demanded for the nurse. It is obvious that the indebtedness to Dean Winternitz for the knowledge with which the graduates of the Yale School of Nursing can go forth to render their service can never be estimated.

The Dean can never forget an episode concerning the physiology course when she asked Dr. Fulton who would give this course. He replied: "My men are so highly specialized, that to give the course you need for your students, I shall have to give it myself." This recalls an interesting incident. Since a number of these students were college graduates, the lists of courses they had already taken in the various sciences were given the instructors with the understanding that the instructor and the student would confer. If they felt that the course had been covered, the time so released could be assigned to another subject. Upon inquiring of a student as to her decision concerning her course in physiology, she replied, "I shall certainly continue, for I would never think it was the same subject I took in college."

Never before was such knowledge available for nursing; never before was knowledge being poured out so unceasingly for the production of a healthy nation.

We are living in an age of change, and thus we may question anyone who speaks with finality. It has indeed been said "absolute certainty is a privilege of uneducated minds and fanatics," for man has today been given a priceless gift—Science—the great exponent of the creative will and

method, radiating energy, fired by an unquenchable curiosity, purified and tempered by life experience.

How impossible to portray with any justice to the subject the superb panorama of the progress of human betterment under the leadership of science through successive generations of ardent devotees, such as Dean Winternitz—the blazing of ever new trails, evolving ever new methods, achieving ever more outstanding results, facing ever new problems, each advance opening new avenues of approach and demanding new types of workers and new expressions of already existing types, until the present opens upon another chaos, the chaos of a disturbed, even distracted world at large. A picture puzzle with some of the pieces already assembled, with the rest scattered inconsequentially on the board, is a perfect illustration of the present situation.

The organizations and institutions concerned in any social project should know themselves to be responsible to, not for, the community they are serving, working through and with the people of the community. Medical education depending upon a free hospital service for clinical experience is neither good medical nor social education, nor is medical education sound that separates mind and body, or social groups. One need not prohibit specialization or decry differing provisions for different incomes. One must posit, in the cause of social economy, that that thoroughness and completeness of service that gives best promise of cure and prevention, and that can be commanded by the rich, is more important for the poor.

The evolution of the hospital from an eleeomosynary institution into one patronized by all social groups at various rates of payment has occurred within a half century. The failure to make available the service of the outpatient department in a similar manner accounts in great measure for the unsupervised maternity cases, the unattended minor ills, and the retardation of the practice of preventive medicine.

No heavier indictment can be brought against twentieth century civilization than that what in one case is a most beautiful event, in another is an enduring tragedy. I refer to the birth of a child. The essentials for the attainment of an earthly heaven have been given us again and again throughout the ages by the inspired of every race. There is no country in the world so well equipped as this, our country, through our Government, to embark on this great and greatly needed social project.

The noblest peace army yet conceived is the international health program, the basic principles of which are co-operation and integration—its concern, the creation of a new tradition that begins in the cradle. Individualistic in

function, international in social concepts, the medical and nursing professions sit at the door of science for the latest message concerning the human organism. The nurse, not less than the physician, therefore, must be versed in the language of science and an expert in the ever changing technical skills. Her approach, whether at home or in foreign lands, also demands a human understanding that connotes a cultural foundation.

As a public servant she should prepare herself for the responsibilities of citizenship; as both nurse and teacher she should seek to interpret her profession, now expressed in many ways, through the branch in which she can achieve ever greater efficiency; her objective, to make this beautiful earth a safer and happier world for the generations to come. When the women of the world awaken to their social responsibility and their power, not through force but through science, destructive methods of achieving greatness will be abandoned. Dean Winternitz opened wide to the nurse the doors of knowledge, so that she might go forth, scientifically and culturally prepared, to take her place in the health program.

Again I protest, my feeble pen can in no wise tell the story of this creative mind's contribution to humanity. How truly has a great scientist* spoken, and in speaking contributes to the assertion that the medical profession holds in its hands the better world we dream of: "Time, creative thought and logic,—they await the convenience of none nor the love of things held sacred; agony attends their course, yet their work is the increasing glory of the world. The growth of psychic life, the enlargement of knowledge, the emancipation of man."

Of Milton Winternitz it should be said, "He will be religious and he will be patriotic: 'to do good' will be his religion, and his love of country will embrace the world. For he will be the scientific organizer and director of the civilizing energies of the World in the interest of all mankind."

* Cassius Jackson Keyser, "Mathematical Philosophy."

THE HETEROLOGOUS TRANSPLANTATION OF HUMAN MELANOMAS*

HARRY S. N. GREENE

Following the observation that human cancer possesses the ability to survive and grow in animals of lower species, a considerable number and variety of tumors have been studied with respect to this attribute.[1] The tumors can be maintained by serial passage in the new species and, being borne by animals amenable to experimentation, provide a unique material for investigation.[2,3,4,5,6] A further approach to the cancer problem of more immediate clinical interest concerns the fact that heterotransplantability is a developmental acquisition and not a property of tumors from their inception. The attainment of the property is of significance in the natural history of the tumor and the success or failure of transfer relates directly to the subsequent fate of the patient.

The results of this study will be presented in a series of papers. The present report will be limited to data derived from the transplantation of melanomata.

Materials and methods

The present report is based on the transplantation of melanomas from eleven different patients. Operative specimens were obtained at separate times from two of the patients and the series consists of thirteen transplantation experiments.

Guinea pigs were used exclusively as recipients. The technique of transfer has been described briefly elsewhere but its apparent failure in other hands suggests that a more detailed account of procedure might be given.[2] The animal is immobilized on an operating board with tie strings in such a manner that the desired eye presents to the operator. The cornea is anaesthetized by contact with a 5% aqueous solution of cocaine administered with a medicine dropper. An incision is made at approximately the midpoint of the upper border of the limbus utilizing a sharp double-edged corneal knife. The knife is directed slightly forward so that the blade enters the anterior chamber without damage to the iris. The pressure necessary to pierce the cornea is sufficient, even with a sharp knife, to rotate the eyeball beneath the lower lid and conceal the operative field. A short, quick thrust of the knife, however, results in an

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adequate opening and the temporary obscurity is inconsequential. Withdrawal of the blade is accompanied by the escape of a small amount of aqueous humor but, unless the iris has been cut by a misdirected knife, there is no bleeding.

Transfer of the tumor is effected by means of a trocar commensurate in diameter with the corneal incision. The trocar should be equipped with a tight-fitting plunger and a short bevelled mouth with all edges filed to smoothness. A small fragment of tumor is placed in the mouth of the trocar and forced into the barrel, a step greatly facilitated by withdrawing the plunger to produce suction. The tip of the trocar is inserted through the incision a short distance so that the bore is entirely within the chamber and the fragment is expelled. It is important that all manual pressure about the eye and head of the animal be released before withdrawing the trocar to prevent escape of the fragment. Finally, the fragment is forced into the inferior angle of the iris by applying light pressure along the corneal surface with a blunt instrument. The incision is not closed.

The procedure is executed with great rapidity and the technique is extremely simple. Yet, some workers have encountered difficulties and several points warrant further emphasis. The knife should be sharp and of such width that a stab wound will admit the trocar. The use of a narrow knife necessitates side cutting to obtain a sufficient opening and this may be attended by iris damage with consequent bleeding. Pointed surgical blades or single-edged corneal knives are undesirable, for the triangular cut produced heals slowly and may result in herniation of the iris.

The fragments of tissue to be transplanted should be cut from the tumor mass with sharp instruments and should not exceed 1 mm. in diameter. The selection of fragments is by far the most important step in the procedure. In addition to essential parenchyma, all human cancers contain stroma and many are infiltrated with desmoplastic connective tissue. Such tissues are adult in nature and will not grow on transfer. In fact, their presence in quantity will give rise to a foreign body reaction in the alien host and result in death of the transplant. It is essential, therefore, that selection be based on the content of tumor parenchyma and some knowledge of gross pathology is necessary for such a differentiation. Frozen sections obtained from different areas of the tumor mass are of considerable aid in some cases. It is obvious that necrotic tumor or normal tissue adjacent to the tumor are not suitable materials for transfer.

The placing of the fragment in a wedged position in the inferior angle of the iris is important for early vascularization. Occasionally, fragments so placed work loose shortly after operation and an examination with the required readjustment is desirable before returning the animals to the colony. Fixation occurs within a few hours and further check is unnecessary.

The tumor tissue may be transplanted immediately after operation or stored at ice box temperature for several days before use. It is essential that the material be free of infection and that surgical sterility be maintained throughout all manipulations.

The animals are not subjected to special treatment after operation, but are carried under the ordinary conditions of cage life. It should be emphasized that guinea pigs are incapable of synthesizing vitamin C and require a daily measure of greens. Transplanted tumors grow poorly if at all in ill-kept, inadequately nourished animals.

The melanomas used for transfer were biopsy specimens obtained from adult individuals at the New Haven Hospital. They represent a small portion of a large series of tumors subjected to transplantation during the years

1943 to 1950 and include all of the melanomas received from the operating room in suitable condition for transfer. No attempt was made to transplant tumors that had ulcerated in the patient or were sent to Surgical Pathology in unsterile containers or in formalin. Material obtained by needle biopsy was also rejected. One tumor (No. 7) was transplanted in 1939 at the Rockefeller Institute for Medical Research in Princeton, New Jersey, having been transported from New Haven by mail in a thermos jug.

TABLE 1

THE RESULTS OF HETEROLOGOUS TRANSPLANTATION OF HUMAN MELANOMAS

Series no.	*Patient*	*Primary site*	*Transfer*			*Present status of patient*		*Survival months†*
			Date	*Tissue used*	*Result*			
7	F.H.	Nose	2/25/39	Node in chest wall	+	Died	9/26/39	7
51	R.D.	Foot	3/3/43	Inguinal node	+	Died	6/28/44	15
53	E.C.	Vulva	3/15/43	Inguinal node	—	Died	11/11/45	30
64	F.W.	Shoulder	4/27/43	Primary	—			
207	F.W.	Shoulder	10/26/46	Local Recurrence	+	Died	12/15/46	2
144	J.K.	Face	1/24/45	Cervical node	+	Died	7/16/45	6
151	V.H.	Eye	3/7/45	Primary	—	Alive		
152	A.T.	Eye	3/14/45	Primary	—	Alive		
441	W.U.	Scalp	4/1/49	Popliteal node	—*			
512	W.U.	Scalp	9/30/49	Cervical node	+	Died	1/14/50	3
457	A.H.	Arm	5/10/49	Axillary node	—	Alive		
462	E.W.	Ear	5/20/49	Cervical node	+	Died	6/22/49	1
536	D.H.	Back	12/2/49	Axillary node	+	Alive		

+ Indicates successful transfer.
— Indicates unsuccessful transfer.
* Tumor failed to grow in normal environment of anterior chamber but did grow when transferred in contact with placental transplants.
† Survival periods are computed to the date of writing, January 20, 1950.

Results

The results of the transplantation experiments are shown in Table 1. The melanomas borne by seven of the patients were successfully transplanted to guinea pigs, while transfers from four other patients were unsuccessful.

A comparison of the results of transfer and the fate of the patients shows the existence of a direct relationship between transplantability and the stage of the disease at the time of operation. One of the individuals with a transplantable tumor is alive at the time of writing, one month after transfer, but there is definite clinical evidence of liver metastasis. The remaining six patients all died within 15 months of successful transfer, the survival periods

being 1, 2, 3, 6, 7, and 15 months. In contrast, three of the four individuals with non-transplantable tumors are living; two have survived for nearly 5 years; one, A. H., Case 457, is clinically free of tumor seven months after an axillary dissection. The fourth patient of this category died thirty months after a negative transfer, a survival period twice that of the longest living patient with a transplantable tumor.

In two instances, successive biopsy specimens were obtained and the results of transplantation are of special significance as illustrations of the developmental progress of the tumor in the interim between operations. In one case, F. W., the primary tumor in the skin of the shoulder was removed in April, 1943, and transfer was unsuccessful. Three and a half years later, the patient returned for removal of a local recurrence and, at this time, transfer was successful. The significance of these results in relation to the developmental stage of the disease is indicated by the fact that the patient died with metastases one month after the positive transfer.

The second case, W. U., is similar in pattern. A popliteal mass was removed in April, 1949, and representative fragments failed to grow on transfer to normal guinea pigs. In contrast, a cervical node removed in September of the same year was readily transplanted and the patient died with metastases three months later. It is of interest in passing to note that although the specimen obtained in April could not be tranferred to normal guinea pigs, growth was obtained when the tumor fragments were transplanted in association with fragments of guinea pig placenta. The significance of growth under such circumstances is not clearly understood but similar results have been obtained with other tumors and it appears to be a general rule that tumors on the verge of becoming autonomous will grow in association with transplants of embryonic tissue although they never survive when transplanted alone. The growth of placenta under the conditions of the experiment is in itself worthy of note. Despite numerous attempts, it has not been found possible to transplant isolated fragments of placenta, but good growth is obtained when the tissue is transplanted in combination with embryonic organs. The point of interest in the combined transplant in question is that the melanotic tumor apparently exerted an influence comparable to that of embryonic tissue and supplied the conditions necessary for placental growth. The finding is an incidental observation and has no special application in the present discussion. The point to be emphasized is that at one period of development the tumor would not grow in normal animals, while at a later period, antedating death by three months, growth was readily obtained.

A complicating circumstance of possible significance to an interpretation of this case concerns the status of the patient at the time of the first transfer and should be noted. The patient was an elderly individual in considerable discomfort and neither surgery nor X-ray therapy was of promise from a palliative standpoint. In view of the altered behavior of melanomas occurring before puberty, it was felt that some benefit might be derived from castration. After bilateral orchidectomy, there was symptomatic relief and the popliteal tumor actually diminished in size over a period of several weeks. Two months after castration, surgery seemed advisable and the mass was removed. This constituted the material utilized in the first transfer. It is of some interest that, although a needle biopsy performed before castration revealed an amelanotic medullary tumor, sections of the surgical specimen were heavily pigmented and contained an abundance of desmoplastic connective tissue. The material obtained by needle biopsy was insufficient in amount to allow both morphological study and transfer and unfortunately its transplantability was not determined. The point at issue is whether or not the transplantability of the tumor was affected by the change of status incident to castration and an answer is of importance both to an interpretation of the experiments and to an understanding of the biological relationships of the tumors. It is hoped that future studies will yield pertinent data.

The behavior of melanomas in the anterior chamber of the guinea pig differs in several respects from that of other human tumors. Transplants of ordinary epidermoid or adenocarcinomas show evidence of growth in from one to two weeks, but it is not uncommon for a fragment of melanoma to remain unchanged in size or appearance for as long as three months after transfer. Subsequent growth may be rapid so that the chamber is filled during the following two weeks, but, in other cases, it is extremely slow and a period of six months may be required for the tumor to reach a comparable size.

The rate of growth in the pig's eye is apparently without significance from a prognostic point of view. In the case of F. W., who survived successful transfer by seven months, the average period required to fill the chamber was six months, while the patient, R. D., whose tumor filled the chamber in four months, survived for fifteen months. The slowest growing tumor of the series required seven months to fill the chamber and this was derived from the patient, E. W., who died one month after transfer.

The incidence of takes also varied within wide limits and, like the rate of growth, appeared to be without prognostic significance. The highest incidence (six takes among eight animals) occurred in the transfer of the

tumor from R. D., and the lowest (two out of eight) resulted from the transplantation of the tumor from E. W. Notwithstanding, R. D. survived for nearly a year and a half, while E. W. died in a month.

The incidence of takes in this, as in other tumor transplantation studies, bears a direct relationship to the stromal and desmoplastic content of the tumor and depends to an extent on the care exercised in the choice of fragments. Despite careful selection, however, medullary cancers invariably grow in more animals than do scirrhous tumors of comparable autonomy. Second generation transplants of all tumors, whether medullary or scirrhous in the human patient, show much the same degree of cellularity, and the connective tissue content as well as any included normal tissue is, of course, guinea pig in identity. This would seem to account for the fact that such transplants grow in a uniformly high percentage of animals. In the second passage, the tumors of both R. D. and E. W. grew in eight of the ten animals used.

It is probable that more takes would have been observed if the animals of the first generation could have been held for a longer period of time. But, when large numbers of tumors are being tested, it becomes impracticable to hold animals for long-term observation without extensive caging facilities. In the present series of experiments, animals with no evidence of growth were released six months after transfer. In one such case, an animal had been shifted from the experimental population to the breeding colony, and a full year thereafter, growth of the transplanted melanoma was noted. The factors governing the long survival of fragments in a living state, but without any manifestations of growth are of great interest in view of an apparently similar situation in human patients where metastases may behave in an identical manner. The transplanted fragments remain translucent in sharp contrast to the opaque white appearance of dead grafts, and there is no evidence of vascularization until shortly before growth occurs. It would seem that the life of the transplant can be maintained by the simple exchanges possible in the aqueous humor but that actual growth depends on the added nutrition attending a blood supply. The conditions concerned in vascularization of the transplant would thus appear to be of considerable significance in determining the incidence of takes, and whether these are primarily functions of the transplant or of the recipient host is the subject of continued study. It is of interest in passing to note that the rate of growth is subject to changes even after vascularization and that these changes correspond to alterations in the endocrine status of the host. Thus, growth ceases during pregnancy to recur with increased vigor at the inception of lactation.

All of the guinea pigs of the first generation were killed for retransfer or histological study as soon as the period of time required to fill the anterior chamber had been determined. Tumors of the second and subsequent serial generations grew more rapidly and representative animals were held for further observation of the course of growth. In the majority, regression occurred after the tumor had filled the chamber. This proceeded rapidly and the transition from living tumor to complete necrosis generally occupied less than a week. Such animals proved refractory to reinoculation of the tumor in the anterior chamber of the opposite side.

In a few cases, growth continued beyond the point of filling the chamber, leading to rupture of the cornea and protrusion of the tumor in an unsightly fungating mass. This was followed eventually by infection, haemorrhage, and death. At autopsy, there was no evidence of lymphatic extension or metastasis, but in one animal, histological sections of the lung showed thrombi containing cells identical with those of the anterior chamber transplant. It should be emphasized that the cells were confined within blood vessels, and in the absence of extravascular growth, the lesion cannot be regarded as a metastasis.

Histologically, the tumors used for transfer covered much of the architectural range characteristic of melanomata, and with a single exception, the transplants closely resembled the parent growth. The exception occurred in the case of J. K., where the parent tumor was of a solid alveolar type and the transplant fascicular and perithelial in arrangement. The transplantable and non-transplantable tumors showed no distinguishing difference in architecture or cytology and there was no morphological distinction between consecutive biopsies of the same tumor taken during non-transplantable and transplantable phases.

Discussion

The development of heterotransplantability in the natural history of a melanoma is an event imbued with clinical significance. It reflects the acquisition of new tumor properties conducive to rapid dissemination and portends a fatal termination. The fact that heterotransplantability is a function of development and not an inborn attribute of this tumor is of equal import. Despite an identity in histological appearance, the melanoma with lethal metastases is not the same tumor as the neoplastic growth initiating the disorder.

The ability to grow in alien species was demonstrated to be a characteristic of the melanomas in all but one of the fatal cases. The exception constitutes evidence of the developmental nature of the property for the perti-

nent transfer was carried out at a relatively early stage of the disease, antedating death by 30 months. It seems highly probable that had another test been performed at a more advanced stage, a positive result would have been obtained. Such was the case in the patients F. W. and W. U. In the former, a test carried out 3½ years before death gave negative results while a subsequent transfer, 40 months later or two months before death, established the presence of the acquired property. The case of W. U. is co nplicated and difficult to interpret on the basis of present knowledge. However, if only the results in normal animals are considered and the possible effects of castration are disregarded, then takes were not observed in a test performed ten months before death, while transfer seven months later was followed by growth.

More definitive time relationships in the development of heterotransplantability require studies of successive biopsies repeated at frequent intervals and such were not available. In the series as a whole, the survival periods of patients who eventually died of the disease averaged 5½ months after successful transfer and 27 months when transfer failed. But these figures were not derived from chronologically arranged transfers and are of value only as indications of the duration of the changed status of the tumor.

The two ocular melanomas failed to grow in guinea pigs at the time of exenteration and the patients are living and free of tumor at the present writing (approximately five years). Apparently, both of these tumors were completely removed before any dissemination had occurred. Such was not the case in the patients F. W. and E. C. whose tumors also failed to grow at the first transfer. The primary growth in the shoulder of F. W. could not have been adequately removed at the first operation, for it recurred locally 42 months afterwards. The primary tumor did not grow on transfer but the tumor cells left in the patient continued their biological development and during the 3½-year interim between operations attained heterotransplantability. The case of E. C. presents a different picture. The inguinal node removed at operation was clearly not the only one involved, and tumor cells in other foci subsequently underwent development to biological autonomy and killed the patient.

The significance of failure to grow on heterologous transfer has been studied extensively in the tumors of lower animals and it has been demonstrated that the properties of heterotransplantability and metastasizability are either one and the same or are attained simultaneously. Thus, failure to grow on heterologous transfer is interpreted to mean that the tumor is not yet capable of metastasis. This conclusion was drawn from

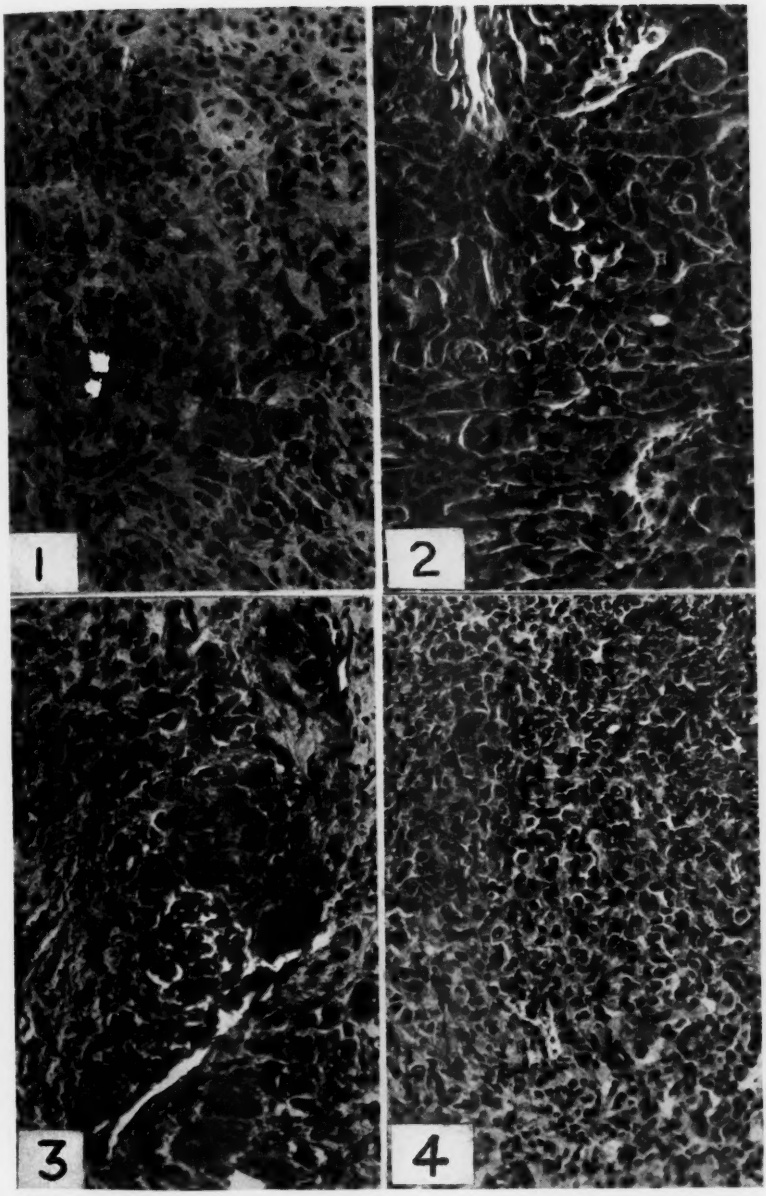

FIG. 1. F.H. (No. 7) Tissue used for transfer. All sections stained with H. & E. All mag. x200.
FIG. 2. F.H. (No. 7) Transplant from anterior chamber of guinea pig.
FIG. 3. R.D. (No. 51) Tissue used for transfer.
FIG. 4. R.D. (No. 51) Transplant from anterior chamber of guinea pig.

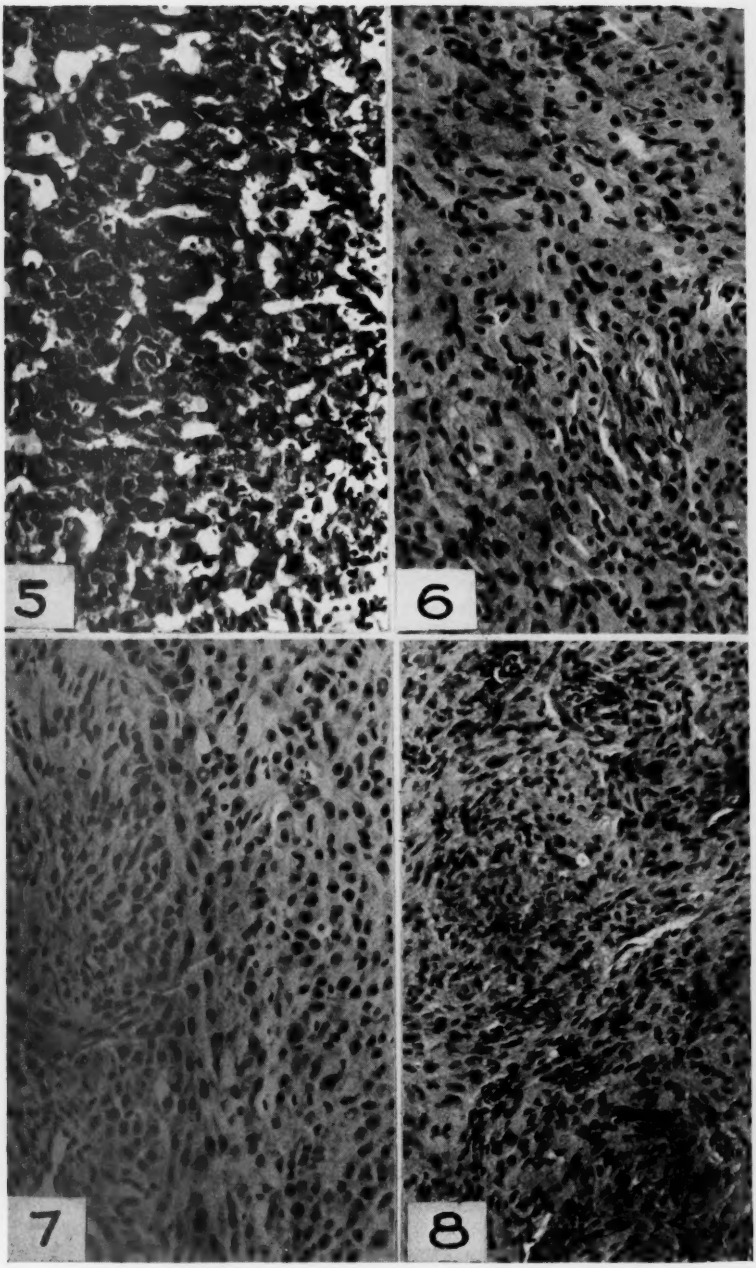

FIG. 5. E.C. (No. 53) Tissue used for transfer. No takes were obtained in the guinea pig's eye.

FIG. 6. F.W. (No. 64) Tissue obtained from primary tumor in April, 1943. Guinea pig transfer was unsuccessful.

FIG. 7. F.W. (No. 207) Tissue obtained from recurrence of previous tumor in October, 1946. Transfer was successful.

FIG. 8. F.W. (No. 207) Transplant from anterior chamber of guinea pig.

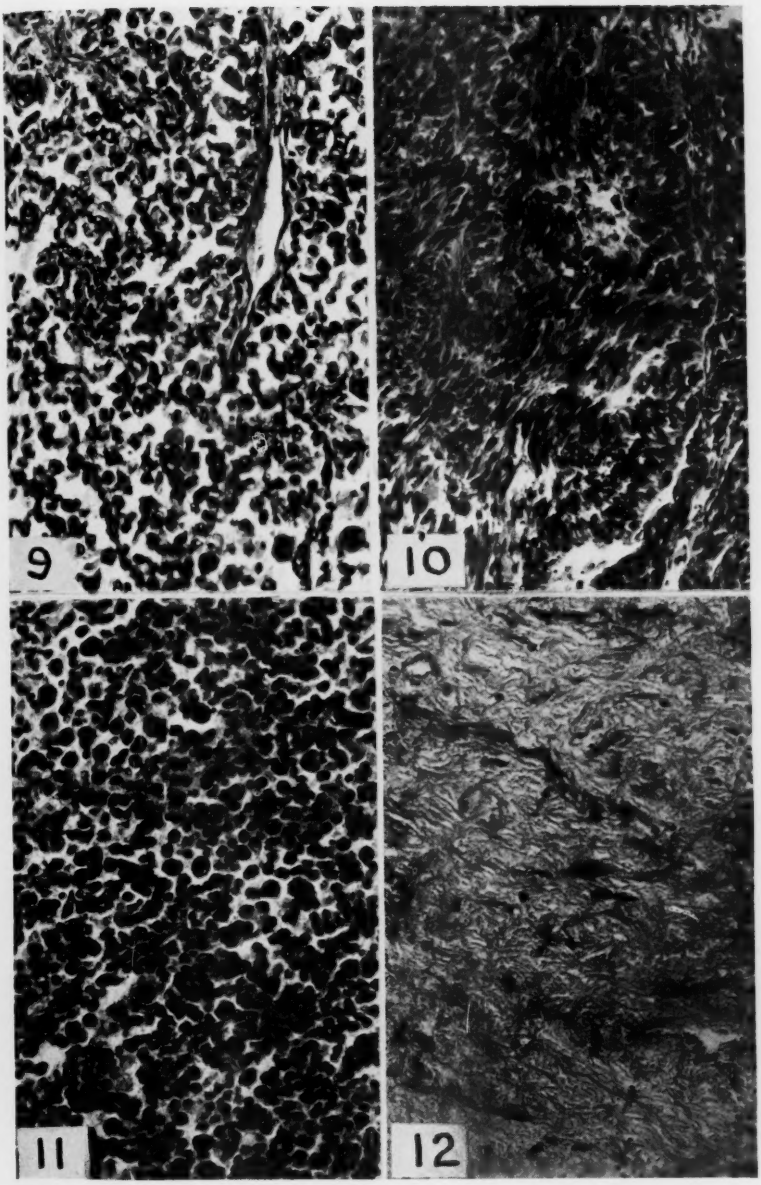

Fig. 9. J.K. (No. 144) Tissue used for transfer.

Fig. 10. J.K. (No. 144) Transplant from anterior chamber of guinea pig. Note change of architecture from solid alveolar to fascicular perithelial pattern.

Fig. 11. A.H. (No. 457) Tissue used for transfer. No takes were obtained.

Fig. 12. A.H. (No. 457) Histological appearance of residual tissue in anterior chamber 2½ months after transfer.

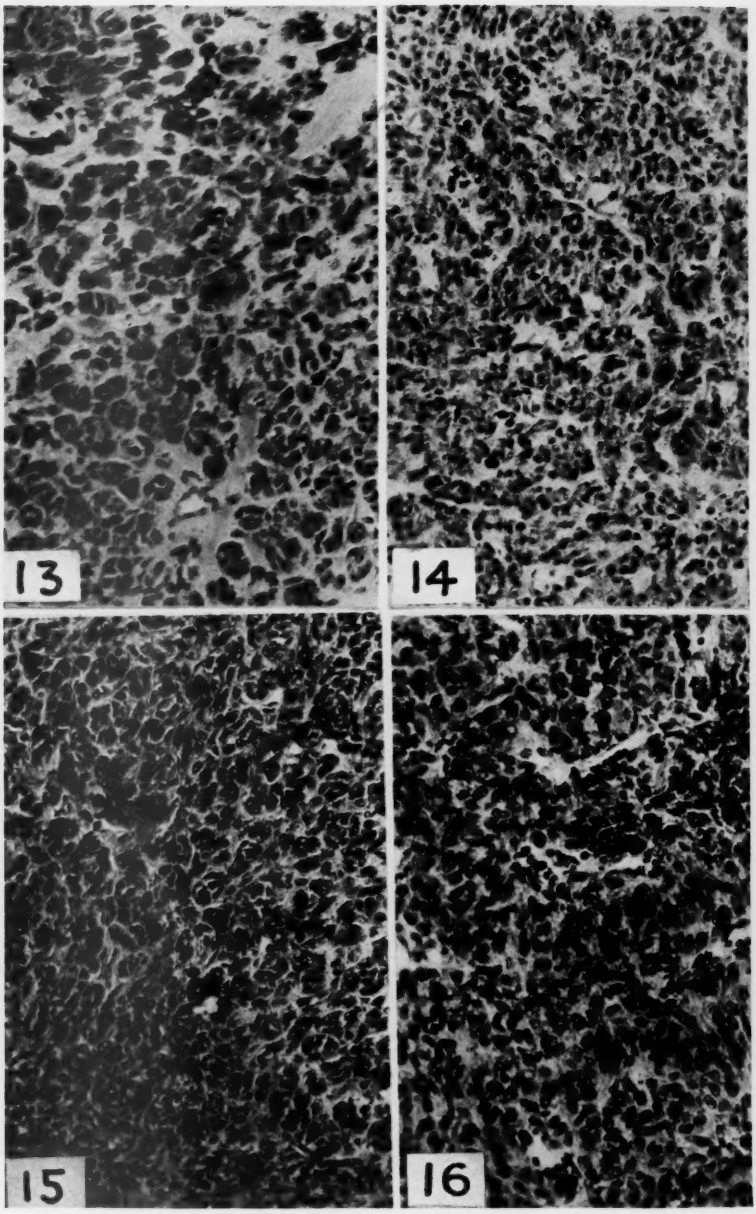

FIG. 13. W.U. (No. 441) Tissue obtained from popliteal mass in April, 1949. No takes resulted in normal pigs but growth occurred in transplants combined with fragments of placenta.

FIG. 14. W.U. (No. 441) Transplant from pig's eye growing in association with transplant of placenta.

FIG. 15. W.U. (No. 512) Tissue obtained from cervical node of same patient in September, 1949. Transfer to normal pigs was successful.

FIG. 16. W.U. (No. 512) Transplant from anterior chamber of normal guinea pig.

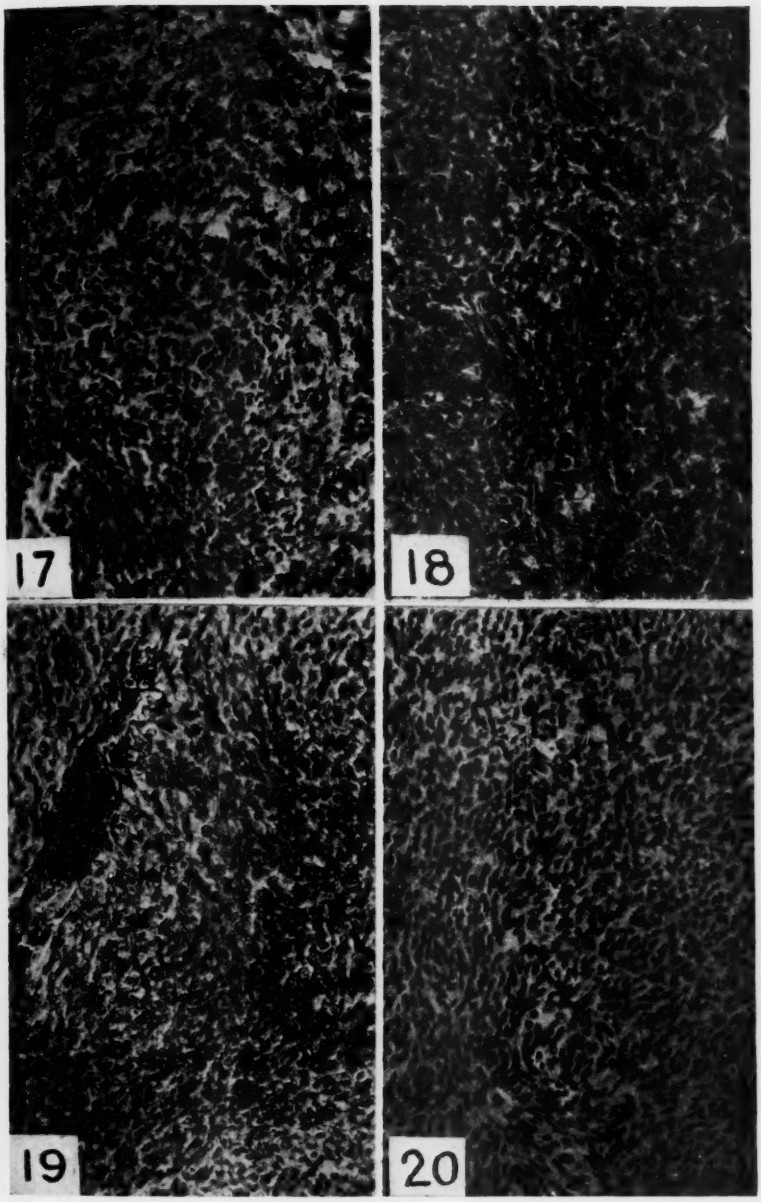

FIG. 17. E.W. (No. 462) Tissue used for transfer.
FIG. 18. E.W. (No. 462) Transplant from anterior chamber of guinea pig's eye.
FIG. 19. V.H. (No. 151) Tissue used for transfer. No takes were obtained.
FIG. 20. A.T. (No. 152) Tissue used for transfer. No takes were obtained.

careful post-mortem examination of the bodies of animals for the presence or absence of metastases after the transplantation of their tumors. Such examination is obviously not possible in man and the conclusion is largely inferential as applied to this species. It has been found, however, that human tumors with recognizable metastases always grow on transfer and, in a number of cases, the unsuspected presence of metastases was first disclosed by positive transfer and later proved by x-ray. Conversely, the survival periods of patients with non-transplantable tumors are such as to exclude the possibility of metastases being present at the time of transfer. It should be emphasized that, in these considerations, lymphatic extension is sharply differentiated from metastasis. Secondary growths arising from lymphatic extension of a primary tumor, e.g., the great bulk of axillary nodes in breast cancer, are not transplantable and neither the primary nor secondary growths become transplantable until the ability to form true discontinuous metastases is attained.

The present data indicate that melanomas are comparable with other tumors in these respects. The inguinal node in the case of vulval melanoma (E.C.) and the axillary node derived from the melanoma of the arm (A.H.) failed to grow in guinea pigs and probably represented lymphatic extensions of the nearby primaries. E. C. lived 3½ years after removal of the inguinal node and at present, 8 months after axillary dissection, there is no clinical evidence of tumor in the patient A. H. In contrast, it is suggested that the nodes successfully transplanted to guinea pigs were either true metastases or lymphatic extensions of tumors that had metastasized elsewhere. The average survival period of 5½ months in such cases is supportive evidence.

A paradox appears when such considerations are applied to the case of W. U. The secondary focus in the popliteal node could hardly have arisen as a lymphatic extension of the lesion in the scalp. Yet, its behavior was not that of a metastasis for it did not grow on transfer to normal animals. A speculation, worthy of further inquiry, is that the popliteal node was actually a metastasis and that the biological constitution of the tumor including its transplantability was modified by castration. Such a modification is known to follow castration in prostatic cancer and is manifest by the temporary failure of new metastases to appear and the regression of existing lesions. It is conceivable that the relationship between the failure of the prostatic cancer to metastasize and of the melanoma to transplant may rest on an alteration of the tumor cell affecting its ability to elicit stroma. In such cases, successful transfer of the melanoma would be expected if the

transplanted tumor could be supplied with a ready-made stroma. In actuality, takes did occur when fragments of placenta were added to the tumor inoculum and the function of the placental tissue in this outcome may well have been purely stromal and supportive.

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A NOTE TO DR. WINTERNITZ

Among the medical schools responsible for the surge forward of American Medicine in the first third of this century, the Yale Medical School has taken a special place. More than anyone you are responsible for the accomplishments and the continuing intellectual tradition of that school. To an admirable degree you have justified the trust placed in you that you would insist on putting your convictions regarding medical education into active and uncompromising application. Few deans of your generation had as clear a formulation of the importance of the intellect in medical education. None was as passionately determined to reform the medical school so as to produce doctors who could meet the need, not merely the demand, for comprehensive and comprehending medical care.

You were a ferment where the proprieties could readily have sterilized an environment apparently reconciled to complacency. On top of regeneration and reform, your energy pressed further innovations, unremittingly. Relentless in criticism, tireless in enthusiasm, insistent on action, and unequalled in conviction, you gave yourself as well as the Yale Medical School no rest, no peace, and no counterfeits or excuses. I shall never stop admiring your fiery devotion to the best there is—human and superhuman.

ALAN GREGG

THE NON-UNIVERSITY TEACHING HOSPITAL AND POSTGRADUATE MEDICAL EDUCATION*

JOHN C. LEONARD

The evolution of postgraduate medical education has been a most interesting one. If one remembers that before 1900 it was quite possible for a person of average intelligence with a diligent attitude toward his work to encompass rather completely the known field of medicine and that since then increasing specialization has been necessary because of the rapidly broadening horizon of medicine, many of our present-day problems in postgraduate medical training are more understandable.

Before 1900 only a small percentage of medical school graduates either felt the need of postgraduate training in the form of an internship or were forced by state law to serve an internship. Today, even though an internship still is not demanded in some states, practically 100 per cent of medical school graduates serve such a period of postgraduate training and some medical schools do not award the M.D. degree until the end of this period of training.

World War I gave added impetus to specialization and since 1920 a constantly increasing number of residencies, superimposed upon the internship training period, have been necessary in order to provide men thoroughly trained in the specialties of general surgery and its subspecialties, internal medicine and its subspecialties, obstetrics and gynecology, pediatrics, neurology and psychiatry, etc.

Before 1920 the great majority of interns were trained in university hospitals under the close supervision of the faculty of the medical school; since 1920 an increasing number have been trained in non-university hospitals where their supervision and training ranged from good to indifferent to poor, depending upon the quality and interest of the hospital staff. Before 1930 there were more medical school graduates annually than there were good internships available; but gradually (between 1930 and 1940) an even larger number of hospitals were approved for intern training (many of which in retrospect had inadequate staff and facilities for intern training) with the inevitable result that now we have approximately 5,600

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new medical graduates and 9,370 approved internships from which they may choose their place of training.* Obviously then, a former "sellers' (hospitals') market" has now become a "buyers' (interns') market" with the intern having a wide choice of hospitals in which to serve. It is understandable that the intern should choose for his period of postgraduate training either the university hospital where traditionally he may expect to receive good supervised training or the non-university hospital which has put considerable time, interest, thought, and effort into a carefully planned program of medical education for the house staff.

World War II, with its concentration of attention upon the medical and surgical specialties, gave additional impetus to specialization; and many young men who formerly had been in the general practice of medicine felt both the theoretical and economic urge to become trained in a specialty in the immediate postwar period. This was made considerably less difficult for them economically by the financial assistance which was provided for them by federal aid in the form of subsistence allowance and tuition. Thus, residency training programs have grown so rapidly that there are now more than 18,000 appointments annually; and, since the financial aid of Public Law 346 (G.I. Bill of Rights) is now decreasing, more than 1,000 of these residencies are vacant at the present time. With the gradual decrease in demand for these specialty residencies and an increasing demand for general practice residencies, it is to be hoped that an excessive interest in specialization will be replaced by a normal and increasing interest on the part of medical student, intern, medical school, and hospital in the training of young men for the general, or family, practice of medicine, in which field 85 per cent of total medicine normally exists. This recent and more healthy trend, even though its impetus may be partly an economic one, must be encouraged by our medical schools and our teaching hospitals if it is to produce the greatest possible results. Much of the present deficiency in the quantity of medical care in rural areas can be corrected in this manner. If young men, following their postgraduate training in medicine, can be encouraged to settle in rural and semi-rural areas in groups of two or more, then we shall have further reason for optimism regarding the better distribution of medical care. In stimulating such a movement, both medical schools and teaching hospitals should remember that only by providing for the medical student or graduate a contact with good rural practitioners can such a well-balanced system hope to succeed.

* J. Am. M. Ass., 1950, *142*, 1145.

Only those who have had an opportunity to know and appreciate the joys and rewards of rural or semi-rural medical practice can be expected so to choose their professional location.

Salaries

Before World War II the problem of salaries of interns was not a difficult one since it was generally and traditionally believed that the salary, other than maintenance, was likely to be in inverse proportion to the educational and training value of the internship. The increased competition by the hospitals for available interns, however, has changed this picture. Army, Navy, United States Public Health Service, Federal Security Agency, and Veterans Administration hospitals all offer salaries far in excess of those which can be offered by most university hospitals or by the large group of non-profit community hospitals. This has been considered by many as a decidedly unfair practice, since the same taxpayers are, by added taxes, paying attractively high salaries for interns and residents in government controlled hospitals while the average citizen's own community hospital goes begging for interns whose salary, if provided at all, can only be provided by increasing the cost of hospital care to a still higher level. A compromise in the above discrepancy should be reached.

Living quarters

Comfortable and attractive living quarters have become increasingly essential as part of the competitive struggle for intern and resident staff. No longer can the average hospital provide dull, dingy (and at times even unhealthy) living quarters for its house staff. Tennis courts and a game room have been considered essential for many years. More recently those hospitals which can afford it have added radio, television, a general library, and even a gymnasium, and swimming pool, to further attract available medical graduates. Bachelor quarters have traditionally been expected; but, more recently, in order to remain in competition, some hospitals are willing to provide apartment quarters for the intern or resident and his wife, while a few hospitals have offered living quarters for the intern, his wife and children. This latter point frequently has been influenced in the past five years by the increased demand on the part of the physician-veteran for further hospital training.

Educational program

In former years medical graduates were advised by their medical school to scrutinize closely the post-mortem percentage of the hospital they were

considering for an internship. This is still important and good advice, but now these young men are also warned to look for and inspect carefully the educational and training program of the hospital in which they are interested. The educational program cannot be an irregular, disorganized happenstance. To meet modern demands, it must be a well-organized, seriously thought out, and supervised program designed to meet the needs of the intern and resident in supervision and training. It should contain all of the elements subsequently discussed if it is financially possible for the hospital to sponsor such a plan.

Pathology

The pathology department and its staff play an extremely important part in any house staff teaching program. Formerly the American Medical Association's minimum post-mortem requirement for a hospital was 15 per cent of all deaths. This has been raised to 20 per cent and there is a rather general feeling that it should be raised still higher, since approximately 20 per cent of necropsies are likely to be requested by the relatives of the deceased patient in these medically more enlightened times.

In most non-university hospitals the pathologist is also in charge of the clinical pathology laboratory and, therefore, has an excellent opportunity to instruct and guide the effective development of the young physician. It is quite helpful to all concerned if the pathologist can find time to attend ward rounds occasionally so that he may guide the more efficient use of the laboratory and so that he also may learn on the wards the manner in which the laboratory may be falling short of its goal. It is often quite surprising how an excessive volume of clinical laboratory work decreases if either the pathologist or the attending physician will ask why a particular test or group of tests was performed and what was expected to be learned from the tests.

Clinical pathological conferences

These conferences, in which the clinician and the pathologist combine their forces to present both the pre-mortem and post-mortem findings, are invaluable methods of teaching. This panoramic view of the patient's course provides an opportunity to view both diagnostic and therapeutic errors and triumphs alike and gives the young postgraduate student an excellent stimulus toward better medical achievement. These conferences should be held at least once each month.

Death review

If the death review is conducted properly, it should provide an extremely

valuable teaching device. Large hospitals may find it necessary to have departmental death reviews so that this work may be done with greater efficiency. It is most important that free and open discussion of errors by both the attending staff and the house staff be encouraged if these reviews are to have their full teaching value. These sessions should be held at least once each month and should not be combined with or follow the business meeting of the department or hospital staff. If the death review is combined with the business meeting, the death review almost invariably suffers from the standpoint of time allotted, interest, and teaching value.

X-ray

It is essential that a good x-ray service be available to the hospital if it is to provide adequate house staff training. It is true that many more x-ray films are taken in teaching than in non-teaching hospitals. However, when the number of such films becomes excessive, it usually can be controlled or improved by either the attending physician or the roentgenologist, or preferably both, asking the young postgraduate student, first, the purpose of each and every examination and, second, whether the x-ray film was truly necessary in the diagnosis or treatment of the patient. It also is very important that the roentgenologist be easily available for consultation. If time and the size of the x-ray staff permits, there is great value in having a member of the x-ray department attend all formal teaching sessions.

Medical library

The importance of the medical library to the postgraduate program of the hospital cannot be overestimated. It is essential not only to have a medical library, but also essential that this library be located where it will be quickly and easily accessible so that members of the house staff and attending staff alike may quickly look up a point in diagnosis or therapy or may read a current medical article in a few minutes which otherwise might be wasted because of time-consuming inaccessibility of the library. The medical library of the non-university hospital should contain up-to-date textbooks for quick reference. Out-of-date textbooks, which have no modern value and which are too recent to have even antique value, should not be given shelf space. Systems of medicine or surgery are usually so far out of date that we consider them no longer essential to a medical library. A medical library in a non-university hospital should place great emphasis upon its current medical journals. The library should not be judged by the total number of medical journals available, many of which might be used

rarely or never, but should be judged by the qualitative selection of journals which will be used. Thus forty to sixty journals may be all that will be necessary for the individual library, and a lending system usually can be arranged with a larger and more complete medical library in the area.

It is essential that a medical library be supervised, perferably by a medical librarian, or at least by one who has had some library training. Before we carried out this plan, textbooks and current journals frequently were missing from the library. This has been corrected by the presence of a full-time librarian. Books or journals may be loaned from the library overnight but, unless the library possesses duplicate copies, these should be returned the following morning. Only a few medical libraries can afford the twenty-four-hour coverage necessary to remain open constantly but it may be possible to have the library open from 8 or 9 a.m. until 9 or 11 p.m. with proper auxiliary coverage. The medical stacks also should be under the constant supervision of the librarian since, generally, it has been found that the "honor system" is not effective in maintaining a good medical library.

Medical records

The hospital must maintain an adequate medical records system if the patients' records, past and present, are to have any value in the training of the house staff. The medical records librarian and the medical records committee of the professional staff must be given adequate executive authority to carry out the hospital's rules and regulations concerning the preparation, filing, and review of medical records if good results are expected. Cross-indexing is essential if medical records are to be meaningful for clinical research and it is important to adhere to the use of the Standard Nomenclature of Disease and Operations.

Ward rounds

Ward rounds in a hospital, traditionally, have been synonymous with teaching rounds, but in many hospitals ward rounds have degenerated into a race against time with the attending physician wearing the proverbial track shoes so that he can cover the territory in the least possible period of time. The attending physician must give adequate time to ward rounds so that he may advise and guide the intern or resident not only in diagnosis and therapy, but also in the day-to-day progress of the patient's disease and treatment. Too often in the past, ward rounds have been assigned only to specialists in the various branches of medicine and surgery, thereby overlooking the inspiration and value which may be brought to the young

postgraduate medical student by carefully selected general practitioners (family doctors) in this work. Greater numbers of men will be attracted to the general practice of medicine if this is done in both university and non-university hospitals.

Formal lectures or clinics

It is well to have a carefully organized plan wherein as large a number as possible of both house staff and attending staff participate; but it is also important, at monthly or weekly intervals, to have a stimulating speaker from a medical school or from another medical center. This speaker may be chosen for his ability to present a stimulating subject, or he may present a new phase of medicine or may describe some recent investigative problem or may even talk about some phase of the much neglected "art of medicine. Occasionally it is well to encourage a younger member of the hospital attending staff or an unusually able member of the house staff to present the material of his study before a formal clinic.

Informal clinics and case presentation

These sessions should be pointed toward giving the intern and/or resident an opportunity to present cases and topics so that he may be encouraged to think while on his feet. There is no doubt that this opportunity for the house staff to "learn by doing" has great value. It is recognized widely in educational circles that the one who gains most from teaching others is the teacher himself. Such a supervised plan, with members of the attending staff present to give constructive criticism, constitutes one of the greatest strengths of a teaching program. It is a great joy to watch the progress of an embarrassed, hesitant intern from his first case presentation to the smooth, well-organized presentation of a few months later when he has been encouraged to "learn by doing." In general it can be said that bedside teaching and case presentation form the backbone of any effective teaching program.

Medical motion pictures

During the past decade medical motion pictures have become increasingly important supplements to medical education. These are now obtainable with very little effort and with little or no expense from the American Medical Association, the American College of Surgeons, from individual physicians, and from many of the pharmaceutical houses. Visual methods have always been more totally effective than auditory methods and in the modern medical motion pictures with sound and color together we have an ideal

teaching combination. Television already has been added to teaching methods and undoubtedly it will not be long until it also will become a frequent and important supplementary method in medical teaching. How superior to the old system whereby one was supposed to be able to observe (and perhaps learn) something about surgical technic from the inaccessibly remote operating room gallery!

The hospital bulletin

The hospital medical bulletin or journal has great importance in encouraging the intern or resident to write case reports, medical reviews, and clinical studies. The hospital bulletin should not be looked upon as a contribution to the already over-voluminous medical literature, but should provide a place where the young postgraduate student can publish his early attacks of "pruritis scribendi." The training and inspiration which this provides is helpful in building future ease and self-confidence with medical literature, as well as a greater critical appreciation of the writings of others.

Journal club

The alert staff of many hospitals has its journal club and it often is helpful for the house staff to have its own organization of this sort for the purpose of reviewing and discussing the current medical literature. The house staff journal club should be for, of, and by the intern and resident staff if it is to succeed. All too frequently a member of the hospital attending staff, who is the adviser, takes over the discussion to the point that the journal club loses its inspirational value for the house staff. Large hospitals may find it necessary to have departmental journal clubs if there are many members in each hospital department. The occasional use of medical motion pictures or a speaker, chosen entirely by the members of the house staff journal club, may provide additional interest to the program.

Teaching by the hospital resident staff

It was previously stated that the person who prepares material for presentation and for teaching actually benefits more than those he is attempting to teach. The hospital resident staff, under the constant stimulus of questions asked by the intern staff, benefits greatly. They in turn pass on to the intern staff many usable and helpful points in diagnosis and therapy which might be overlooked by an older attending staff who are likely to assume that these simple but important bits of information are already known to the intern staff. The gap between intern and resident is so slight that interns are likely to ask questions more freely of the resident staff

without fear of embarrassment regarding some point which they perhaps might be expected to know already.

Material for house staff training

It has been accepted traditionally in medicine that ward, or service, patients shall be the material for house staff training. The quality of care afforded these patients has generally been of a very high order since they have the energetic enthusiasm of youth as well as the valuable experience of the attending physician at their disposal. The number of ward, or service, patients, however, has been gradually diminishing during the past decade, as ever greater numbers of patients come under Blue Cross, Blue Shield, and other health insurance plans. It is inevitable, then, that patients of the semi-private and private grouping in hospitals must provide the material for the training of intern and resident staffs in the future. This, obviously, must be done under the constant and careful supervision of the attending staff. It has been our experience that all patients appreciate the greater interest and attention of several physicians in their problems of diagnosis and treatment. There is also no reason why a resident surgeon, after a reasonable period of training, should not be given the position on the right side of the operating table, so long as his attending surgeon takes over the left side of the operating table as his supervising assistant. Certainly an adequate supply of general surgeons and surgical subspecialists cannot be trained without the general adoption of this plan in university as well as non-university teaching hospitals.

Types of house staff training

There is a considerable difference of opinion regarding the "best" method of house staff training.

I. Plan One

This plan provides much didactic teaching for the members of the house staff. The resident may spend several to many years as second assistant at operations and as the "lowest man on the totem pole" may succeed in seeing or learning very little. Periods of specialty training extending from five to ten years are not unusual in this system.

II. Plan Two

This plan of throwing the young medical graduate into work entirely in the "on your own" system without adequate, or at times without any, supervision is dangerous for the patient and provides none of the supervision which is so essential to the proper training of the medical graduate.

In our opinion both of the above systems are to be condemned.

III. Plan Three

This plan combines the best features of I and II so that the intern and resident have the opportunity to "learn by doing" but are under adequate supervision by the attending staff at all times. We feel that a minimum of didactic teaching at this level, with the house staff actively participating in case presentations, clinics, and medical discussions is likely to produce a more alert physician whose first thought is the welfare of his patient.

Intern or house staff committee

The intern or house staff committee in many hospitals is composed of the chiefs of the various hospital services or departments. This, ideally, might be effective, but, all too frequently, the chief of a service may be both too tired and too busy to give this important job the time and effort which it deserves. It has been our plan to try to find the member, or members, of each major department who were enthusiastic about and interested in the problems of the house staff and its training. These men frequently are found in the younger and middle age groups and should be appointed on the basis of their interest and ability, so that the problems of medical education and training will be taken seriously. At the resident level it is usually wise to have each department take care of the problems of selection, supervision, and training of the residents within their own specialty.

Director of medical education

The director of medical education may be the chairman of the intern or house staff committee or a member of this group; he may be the pathologist, and the pathologist is often the ideal man for this position; he may be the radiologist if he has adequate time and interest; he may be a retired, respected member of the staff if he has maintained youthful interest and enthusiasm for this work; or he may be the newly graduated resident if he has the vision, interest, and ability to carry on this type of work.

A few of the larger non-university teaching hospitals are appointing full-time men whose task is the supervision and coordination of the various departmental activities into an overall educational program. Our hospital has had this plan since 1947. Several smaller hospitals within reasonable proximity of each other may secure, among them, the services of a director of medical education who will spend part time in each hospital. Such a plan was advocated in considerable detail in our study of medical training programs in the State of Connecticut in 1944.* It is conceivable that the programs of three or four smaller adjacent hospitals might be coordinated

* Connecticut M. J., 1945, *9*, 131.

by one director of education who could spend part of his time in each of the member hospitals of the group.

Too often in the past it has been theorized that a medical education program aimed at the house staff level would have no interest for, or attention from, the hospital attending staff or from the physicians in the hospital area. This has been proved not to be the case, since the majority of the clinics listed in the Hartford Hospital Medical Education Program (Table 1) are attended by house staff, attending staff, courtesy staff, and by many general practitioners located within 25 to 50 miles of this hospital. It has been estimated that if a physician of this hospital area faithfully attends the various noon clinics held here, he will have, at the end of one year, the equivalent of more than one month's postgraduate course. If, because of the pressure of his work, he cannot attend the noon clinics but can attend the clinics of the Saturday morning program, he will have, by the end of one year, the equivalent of almost one month of postgraduate medical refresher work. All this without spending a prolonged period of time away from his practice and without any cost to him except the time involved in attendance at the clinics!

Conclusion

The two-way system of intern and resident training in non-university hospitals has come to stay. The time is past when non-university hospitals can hope to succeed in either finding or keeping a house staff in return for the very doubtful privilege of allowing the house staff to perform only the menial tasks commonly known as the "scut work." We must build and adequately maintain our house staff medical education and training programs if we wish to have a house staff at all.

By maintaining a good postgraduate program and offering it to all interested physicians in the area, the hospital can efficiently, painlessly, and with great certainty, improve the quality of medical care for its community.

TABLE 1

HARTFORD HOSPITAL MEDICAL EDUCATION PROGRAM
1949-1950

	MONDAY
9:00-11:00 A.M.	Bedside Rounds—Medicine. Ninth floor, Male Ward (N9R) and Female Ward (C9L).
12:00- 1:00 P.M.	*NEUROLOGY-NEUROSURGERY CLINIC. AMPHITHEATER, EVERY OTHER WEEK (ALTERNATES WITH PSYCHOSOMATIC CLINIC).

12:00- 1:00 P.M. *PSYCHOSOMATIC CLINIC. AMPHITHEATER, EVERY OTHER WEEK (ALTERNATES WITH NEUROLOGY-NEUROSURGERY CLINIC).

4:00- 5:00 P.M. X-Ray Conference. Conference Room, X-Ray Department; for radiology residents.

4:45 P.M. *Showing of Surgical Films. Amphitheater, second and fourth Mondays of each month (October through June).

5:00- 6:00 P.M. *Hartford Medical Society Guest Speaker. Amphitheater, first or third Monday of each month (to be announced).

7:30 P.M. *Obstetrics and Gynecology Journal Club. Intern Reading Room, Cheney Library, fourth Monday of each month.

TUESDAY

8:00- 9:00 A.M. *Orthopedic Surgery Grand Rounds (when Dr. Reynolds is on service). Start at X-ray Department, then Conference Room 863.

9:00-11:00 A.M. Bedside Rounds—Medicine. Ninth floor, Male Ward (N9R) and Female Ward (C9L).

12:00- 1:00 P.M. *MEDICAL CLINIC. AMPHITHEATER.

12:30- 1:30 P.M. *Orthopedics Journal Club. Conference Room 863, first and third Tuesdays of each month.

1:30- 2:30 P.M. Gross Pathology Conference. Pathology laboratory.

3:00- 4:30 P.M. *Anesthesia Conference. Amphitheater.

4:30- 5:00 P.M. *Informal Conference, Radiation Therapy. Conference Room, X-Ray Department.

5:00- 6:00 P.M. *X-Ray Conference. Conference Room, X-Ray Department.

5:00- 6:00 P.M. Bedside Teaching Rounds—Surgery. Fifth floor.

8:00 P.M. Dental Department meeting. Conference Room 663, second Tuesday following change in service.

8:15 P.M. *Pediatrics Journal Club. Conference Room 663, third Tuesday of each month.

WEDNESDAY

8:00- 9:00 A.M. *Ear, Nose and Throat Rounds and Conference. Conference Room 663 and Ward (N6R).

9:00-11:00 A.M. Bedside Teaching Rounds—Medicine (Conducted by Dr. John C. Leonard). Ninth floor, Female Ward (C9L).

11:00-12 Noon *Endocrinology Rounds (Conducted by Dr. Sophie A. Root). Out-Patient Department.

12:00- 1:00 P.M. *CHEST CONFERENCE. AMPHITHEATER. WEEKLY. (Conducted by Uncas-on-Thames on the first Wednesday of each month.)

4:00- 5:00 P.M. Informal X-Ray Conference. X-Ray Department, for radiology residents.

5:00- 6:00 P.M. House Staff Journal Club. Amphitheater.

5:00- 7:00 P.M. Microscopic Pathology Conference. Pathology laboratory.

THURSDAY

9:00-11:00 A.M. Bedside Teaching Rounds—Medicine (Conducted by Dr. John C. Leonard). Ninth floor, Male Ward (N9R).

11:00-12 Noon	Dermatology Rounds. Start in Diagnostic Clinic of Out-Patient Department.
12:00- 1:15 P.M.	*MEDICAL DEATH REVIEW. AMPHITHEATER, WEEKLY EXCEPT THE SECOND AND FOURTH THURSDAYS OF EACH MONTH.
12:00- 1:00 P.M.	*PEDIATRICS CONFERENCE. AMPHITHEATER, SECOND THURSDAY OF EACH MONTH.
12:00- 1:00 P.M.	*ORTHOPEDICS CONFERENCE. AMPHITHEATER, FOURTH THURSDAY OF EACH MONTH.
1:00- 2:00 P.M.	*Medical Movies by Departments of Anesthesiology and Medicine. Amphitheater. (To be announced).
2:00- 3:30 P.M.	Neurology Rounds. C7W.
3:00- 4:30 P.M.	*Anesthesia Conference. Amphitheater.
4:00- 5:00 P.M.	Informal X-Ray Conference. X-Ray Department, for radiology residents.

FRIDAY

9:00-11:00 A.M.	Bedside Rounds—Medicine. Ninth floor, Male Ward (N9R) and Female Ward (C9L).
9:30-11:00 A.M.	Teaching Rounds (Conducted by Dr. John C. Leonard). Out-Patient Department.
12:00- 1:00 P.M.	*TUMOR CLINIC. AMPHITHEATER.
1:30- 2:30 P.M.	Gross Pathology Conference. Pathology laboratory.
3:00- 4:30 P.M.	*Anesthesia Conference. Amphitheater. (After January 1, 1950.)
4:00- 5:00 P.M.	Informal X-Ray Conference. X-Ray Department, for radiology residents.
5:00- 6:00 P.M.	Bedside Teaching Rounds—Surgery. Fifth floor and X-Ray Department.
6:00- 7:00 P.M.	*Electrocardiography—Paul H. Twaddle, M.D. Amphitheater. (December 2, 1949, through February 24, 1950.)

SATURDAY

11:00-12 Noon	*HARTFORD HOSPITAL GUEST SPEAKER. AMPHITHEATER.
8:00- 9:00 A.M.	*Orthopedic Surgery Grand Rounds (when Dr. Pike is on service). Start at X-Ray Department, then Conference Room 863.
9:00-10:00 A.M.	*SURGICAL CLINIC AMPHITHEATER.
10:00-11:00 A.M.	*OBSTETRICS AND GYNECOLOGY CONFERENCE. AMPHITHEATER. WEEKLY EXCEPT FIFTH SATURDAY OF THE MONTH.
10:00-11:00 A.M.	*EAR, NOSE AND THROAT CONFERENCE. AMPHITHEATER. FIFTH SATURDAY.
11:00-12 Noon	*Hartford Hospital Guest Speaker. Amphitheater.

*Open to all interested physicians.

John C. Leonard, M.D.,
Director of Medical Education.

THE GENESIS AND FUNCTIONAL IMPLICATIONS OF COLLATERAL CIRCULATION OF THE LUNGS*

AVERILL A. LIEBOW, MILTON R. HALES, WILLIAM HARRISON, WILLIAM BLOOMER, AND GUSTAF E. LINDSKOG

Foreword

Those with the good fortune to be directly under the spell of M. C. W. will not soon forget how frequently and ringingly he uses the word "opportunity." He has become a master at providing opportunity, in his own Department and School, and now in a larger sphere. A few years ago, with the help of the Office of Naval Research, he provided the idea and the means for a collaborative study of pulmonary disease. In encouraging the collaboration of surgeons and pathologists he was striking another blow at departmental barriers that he had always regarded as an impediment to investigation. This progress report covers one phase of the joint effort. Herein, we shall heed the admonition "Respice; Adspice; Prospice." Let us be forgiven if, in the last, we may gaze into the realm of conjecture, rather than upon work accomplished. But, that we not offend him whom we seek to honor, speculation shall be kept separate from fact.

In his Harvey Lecture of 1936, de Burgh Daly[9] stated: "Concerning the significance of the bronchial-arterial system in health and disease, we are still largely in the dark, but that this may be a fruitful field for investigation is unquestionable." Recent observations in several laboratories have to a degree, confirmed his prophecy; still, much darkness remains to be dispelled.

It was known to Galen that the lungs had a double blood supply, but Luschka's[20] concept of the *vasa publica* and *vasa privata* had to await Harvey's elucidation of the course of the circulation. To William Snow Miller[30, 31, 32] is owed much of our knowledge of the vasculature of the lung. A single pulmonary artery slavishly follows and is joined by fascia to each branch of the bronchial tree, but yields it no branches until the first alveoli appear in the walls of the respiratory bronchioles; the pulmonary veins, on the contrary, are as far removed as possible from the broncho-arterial ray. The pulmonary arteries are end arteries; there are no vessels that traverse the lobule to join adjacent branches and there is no confluence of their separate streams except in the capillary beds at the very periphery of the lobule. The bronchial arteries contrast in that, characteristically, even

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though there be but one major trunk from an intercostal or from the aorta to a lung, a plexus is soon formed with at least two major branches, joined by many laterals, distributed within the wall of each bronchus. Thus, the bronchial is the direct antithesis of an end-artery. Normally, communication with the pulmonary artery is only by means of a capillary bed in the walls of the respiratory bronchioles. How much of the blood from the bronchial arteries, if any, normally reaches the walls of the alveoli themselves is not known.

Normally, blood brought to the lung via the bronchial arteries may follow one of two courses in returning to the heart: In the proximal thirds of the major bronchi, some of this blood is brought via the azygous veins to the right auricle; more distally in the bronchial tree, drainage is into the pulmonary veins which thus come to carry small amounts of unoxygenated blood. We have observed in certain lungs, moreover, that even proximally, drainage may be into the pulmonary venous system and that branches of the pulmonary veins may actually extend far out into the mediastinal tissues in company with bronchial arteries, presumably draining tissues supplied by these vessels. It is thus apparent that most of the drainage is into the pulmonary veins.

The bronchial arteries in disease. In chronic disease of the lung in man notable changes take place in the vasculature. It has often been observed[13] that as the capillary bed is reduced by fibrosis, the pulmonary artery may become thickened and restricted in lumen by subendothelial proliferation of connective tissue. Some of the pulmonary arteries, as is common in tuberculosis, may also become thrombosed and recanalized. Less well known, but probably at least as important, are the changes in the bronchial arteries; in fact they have often been mistaken for pulmonary vessels as is apparent in many published illustrations and descriptions. In most types of chronic pulmonary disease the bronchial arteries become markedly enlarged in lumen, although also somewhat varicose and thick-walled. Peripherally, within the diseased tissue they often approach the size of the pulmonary artery at the same level since the latter diminishes much more rapidly in lumen. At the same time, precapillary anastomoses develop between the bronchial and pulmonary arterial systems, but not with the pulmonary veins. The enlargement of the bronchial arteries about tubercles was noted as early as 1845 by Guillot[15] and was demonstrated in various pathological conditions of the lungs by Wood and Miller,[48] who employed a radio-opaque injection mass and roentgenographic technique. In our experience the most striking changes are observed in bronchiectasis where they had not previ-

ously received adequate study. In bronchiectasis, as demonstrated in vinylite bronchovascular casts,[24] the bronchial arteries usually form a dense plexus of sizable, thick-walled, but large-lumened channels, and their anastomoses with the pulmonary artery may have a diameter as large as 2 mm. (Figs. 11 and 12). We have not found evidence of precapillary connections between pulmonary arteries and veins, as suggested by the observations of Prinzmetal[38] and his co-workers, except in congenital "hemangiomas" of the lung. In congenital heart disease with pulmonic stenosis, the plexuses of collateral vessels may become very extensive[18] (Fig. 1). In this condition, the bronchial arteries may directly join pulmonary arteries close to the hilum, while in bronchiectasis, the anastomoses are situated distally in the walls of the bronchiectatic sacs that usually involve branches of the segmental bronchi of the third and fourth orders. In tuberculosis and other chronic fibrosing disease, where much of the alveolated tissue and its rich capillary bed have been destroyed, the bronchial arteries and their anastomoses are less well developed than in bronchiectasis where there is usually less destruction of the parenchyma. In pulmonary infarcts, there is likewise a rich collateral circulation.[3] Carcinoma tissue in the lung also derives its blood supply from the bronchial vessels (Fig. 2), as has been demonstrated by Wright.[44]

Estimation of volume of collateral blood flow. A knowledge of the volume of blood passing through the collateral channels would be of interest in evaluating the effect on the heart. A method has been developed for measuring the collateral circulation in congenital heart disease by the indirect Fick procedure, which involves measuring the CO_2 produced and estimating the CO_2 content of the pulmonary arterial and venous blood by analysis of gas samples obtained from the lungs under appropriate conditions of equilibration. In the hands of Bing[1] and his collaborators it has yielded interesting information in congenital heart disease, often demonstrating collateral flows as high as 2 liters/min./M^2 in children with tetralogy of Fallot. This procedure is technically difficult and has a high error in that collateral flows of less than 30 per cent of the cardiac output cannot be reliably detected,[14] and is not applicable to the diseased lung where collaterals pass through scar tissue and where there is the additional possibility of reverse flow into the pulmonary artery, as will be discussed later.

Consequently, an experimental approach was chosen in order to obtain some estimate, by more direct methods, of the magnitude of collateral flow as it develops. It has been known that a collateral circulation will appear in the lung after interruption of the pulmonary artery.[22, 28, 29, 37, 39, 40] Experi-

ments in this laboratory[2] show that the collateral is already manifest anatomically, and functionally in a markedly increased flow, two weeks after ligation of the left pulmonary artery. A month or more later the collateral is immense (Figs. 3-5). Precapillary communications with the pulmonary artery develop, thus making directly accessible the entire capillary bed of the lung to the blood brought in from the aorta by the bronchial arteries.[25] Proximally the pulmonary artery usually stays open beyond the ligature and can be injected retrogradely in its entirety from the aorta; it is, so to speak, a diverticulum of the bronchial-arterial system. In view of these anatomical findings the very large calculated flows are not so surprising.

The collateral flow is estimated with the animals under sodium pentobartital anesthesia and with a broncho-spirometric cannula in place, from the following formula: $F = \frac{a}{c - b}(100)$ where F is the blood flow in cc./min. of the side whose pulmonary artery has been ligated (the left side in these experiments), a = cc. of oxygen absorbed/minute by the left lung through the broncho-spirometric cannula from a spirometer containing pure O_2, c = the O_2 content in volumes per cent of the blood in the pulmonary vein, and b, the O_2 content of the systemic arterial blood. It was found in ancillary experiments, as would be expected on theoretical grounds, that the left lung would absorb a significant volume of oxygen only if the systemic arterial blood was not fully saturated. This condition is met as a result of the anesthesia and from the partial obstruction of the airway produced by the bronchospirometric cannula. The value "b" is determined by analysis of the femoral arterial blood whose O_2 content is representative of that in the bronchial artery. The value of "c" is assumed to be the same as that of the systemic arterial blood when both lungs were breathing O_2, an analysis performed in each instance during the first part of the experiment. It is obvious from the formula that if c were lower, then the divisor would be smaller, and the calculated flow therefore greater. Also "F" represents only the effective collateral flow, i.e., that blood passing through well-ventilated functional pulmonary substance. For these reasons, F represents a minimal value for the magnitude of the collateral circulation.

When the oxygen uptake of a lung with interrupted arterial flow (the value "a" in the above formula) is measured at intervals after ligation, it is found to rise with time, a statistical trend line having a significant upward slope for at least a year.[2] This is the case despite the fact that such a lung appears to be less efficient than the normal in the transfer of gases across the walls of the alveoli. Observations on this point, using the left lung as a tonometer, were interpreted as suggesting that the permeability of the alveolar membranes may be impaired after ligation of the pulmonary artery,[19] although with ordinary methods the histological structure did not appear to deviate strikingly from that of the normal lung.[35, 37, 38]

The collateral flow as calculated from the formula was found to rise strikingly, often reaching levels in excess of a liter per minute after the fourth month.[2] This represents at least a forty-fold increase over the volume of blood brought to both lungs from the aorta by the bronchial arteries in the

normal dog, as determined by Bruner and Schmidt[4] with the bubble flow meter.

Functional significance of collateral circulation and pulmonary-bronchial arterial anastomoses. That the burden of this collateral circulation must fall on the left side of the heart is at once apparent from a consideration of the course of the blood (Fig. 13). Blood brought from the lung to the aorta is returned, largely via the pulmonary veins, immediately to the left auricle without first passing through the right side of the heart as does all other blood in the aorta beyond the ostia of the coronary arteries. Stated otherwise, the collateral represents a shunt from the aorta to the left auricle. Thus, the output of the two ventricles differs by the amount of the collateral, i.e., the output of the left ventricle in these animals is approximately one-third greater than that of the right.

It remains to be determined how much added work this collateral flow imposes upon the left ventricle. At first thought, considering the low resistance imposed by the capillaries of the normal lung, it would seem that this burden would not be large, despite the great volume of the flow. Yet the situation is analogous to the shunt encountered in peripheral arteriovenous fistulas where the blood volume is known to rise and the heart undergoes hypertrophy.[20] Speculation is, however, no substitute for the experiment that is planned to determine the effect of establishing such a collateral in some small and well-standardized species such as the rat, where large numbers of controls can be employed. It will also be of interest to determine the blood volumes in animals with a large collateral circulation in the lungs.

Returning to the chronically diseased lung in man, where there is also a vastly expanded bronchial arterial circulation, certain similarities and certain differences will be noted in comparison with the experimental dog just described. In lungs the seat of massive fibrosing disease the collateral flow can only be inferred from the vast anatomical development of the bronchial arteries, since much of the collateral is "hidden", i.e., inaccessible to measurement with present methods for the reason that it does not pass through alveolated parenchyma capable of gas exchange. One may expect it to be large from comparison of the size of the bronchial arteries with those in the dogs and with those in patients with tetralogy of Fallot, where estimates of the actual quality of blood carried by these channels are available. Whatever the volume, that portion of blood which arrives from the aorta by way of the collateral vessels and passes through the pulmonary capillaries represents added work for the left ventricle. This burden helps to account for

the hitherto unexplained hypertrophy of the left ventricle often observed in cor pulmonale[38] in the absence of systemic hypertension. The aortic-left auricular shunt may also be a contributing or determining factor in the excessive total blood volumes sometimes encountered in patients with cor pulmonale.[35] Except in congenital pulmonic stenosis or atresia[7] and in a few patients where there had been operative[21, 38] or embolic[6] obstruction of the pulmonary artery, the diseased lungs of the patients under discussion differ from those of the experimental dogs, in that in the former there has also been some destruction of pulmonary substance with consequent obliteration of the capillary bed.

The existence of the high pressure collateral circulation accounts for the hemorrhages, sometimes massive, of bright red, obviously oxygenated blood, often observed in patients with bronchiectasis or other chronic pulmonary disease, as Wood and Miller[43] have pointed out. The large arterial vessels, containing blood under systemic pressure, are often situated within the lamina propria of bronchi, so superficially that renewed ulceration can easily result in their rupture.

It has long been known that even in massive disease of one lung, there may be no desaturation of the systemic arterial blood, an indication that none of the venous blood in the pulmonary artery traverses non-respiring diseased pulmonary substance. One mechanism responsible for this phenomenon involves the anastomoses between the bronchial and pulmonary arteries. It is obvious that here a high-pressure circulation, the bronchial, comes into direct communication with the low-pressure pulmonary arterial system. It is not surprising, therefore, that blood in the pulmonary artery is shunted from the diseased tissue where anastomoses exist into the normal parenchyma where oxygenation will take place. Evidence for this shunting may be obtained during life by angiocardiography in patients with massive chronic pulmonary disease of one side. Thus in a 32-year-old man with severe bronchiectasis of many years standing, involving all segments of the left lung, the radio-opaque material enters only the normal side (Fig. 7). Steinberg and Robb[40] made similar observations in the earliest angiocardiograms although the explanation has been obscure. Mere collapse and immobilization of a lung, as has been done experimentally in the dog in this laboratory* with silk mesh, although it reduces markedly the amount of blood flow, does not prevent opacification of the pulmonary arteries in the angiocardiogram.

* Unpublished experiments.

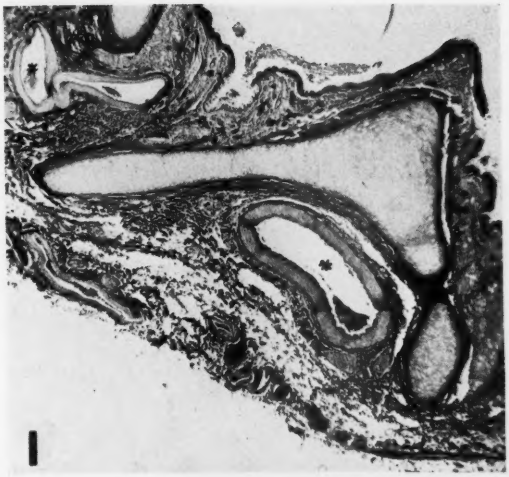
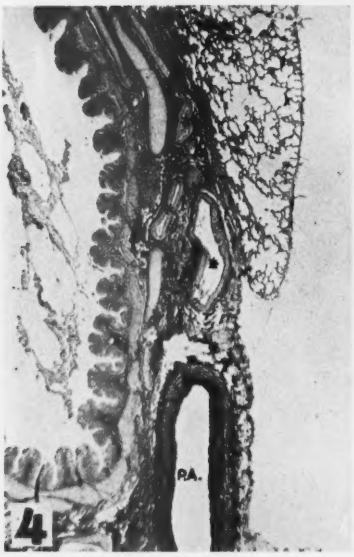


FIG. 1. Histological section of the main bronchus of a patient with tetralogy of Fallot, who died at the age of 17 years. The bronchial arteries are greatly enlarged and have thick muscular walls. Compare with the vessels of the dog shown in Figures 4 and 5.

FIG. 2. Bronchovascular cast of lung with carcinoma arising in wall of tuberculous cavity. Posterior view. The aorta (AO.) curves obliquely downwards and to the right. The pulmonary artery is labeled P.A. The cavity has been filled with plastic seen as the large, almost white, rose-shaped mass (CAV.). About the cavity is shown a tangled mass of bronchial vessels, injected in black from the aorta. They represent the vascular skeleton of the tumor.



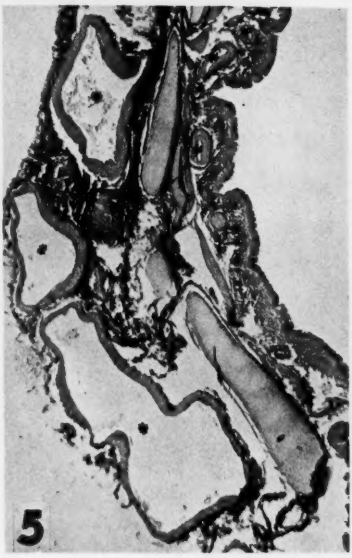
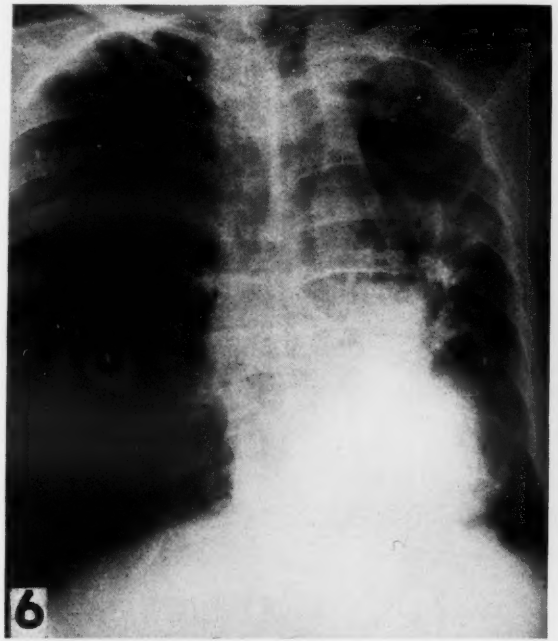


FIG. 3. Bronchovascular cast of twelve-week-old puppy whose left pulmonary artery was ligated and whose right pulmonary artery was constricted with a band of irritating polyethylene, when the animal was less than 48 hours old. There is only a slight increase in the collateral supply of the right lung, while the left displays a tremendous plexus of large bronchial arteries which ramify into the pulmonary substance. The left pulmonary artery has been retrogradely injected through anastomoses with the bronchials at many points on the periphery. The distal segment of the right pulmonary artery (RPA) (beyond the constricting band) is greatly dilated.

FIG. 4. Section of the main bronchus of the right lower lobe (on the side of constriction) of an animal prepared in the same fashion as the one described in the legend of Figure 3, but surviving to the twentieth week. There is a moderate increase in the size of the bronchial arteries*. These are much larger than normal but are not nearly so expanded as they are on the side of complete ligation (Fig. 5).

FIG. 5. Section of the main bronchus of the left lower lobe (on the side of ligation) from the same animal (Fig. 4). The bronchial arteries (marked with an *) have enormous lumina and thick muscular walls.



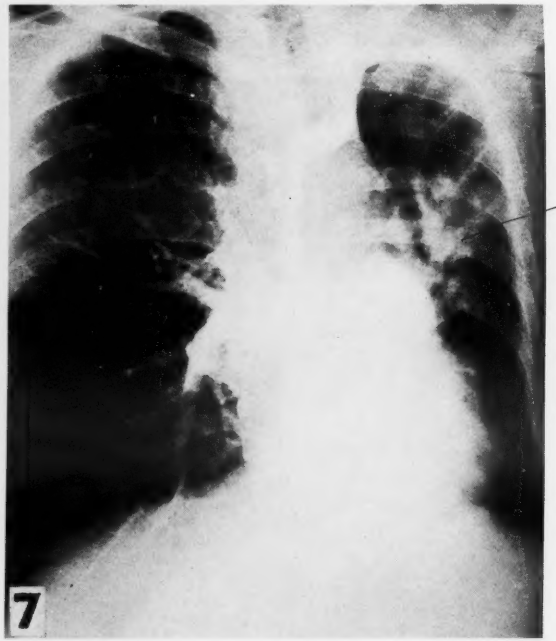


FIG. 6. Roentgenogram of chest of a patient with bronchiectasis of the left lung, made in January 1949. Fibrosis is demonstrated in the left lower lobe, but there is also much radiolucent tissue especially in the left lower lobe, despite the universal bronchiectasis of all segments (Figs. 8 and 9).

FIG. 7. Angiocardiogram, 3½ seconds after injection of diodrast. made on the same day as the film reproduced in Figure 6. The radio-opaque material is seen within the branches of the pulmonary artery only on the normal right side. The left pulmonary artery is "physiologically amputated."

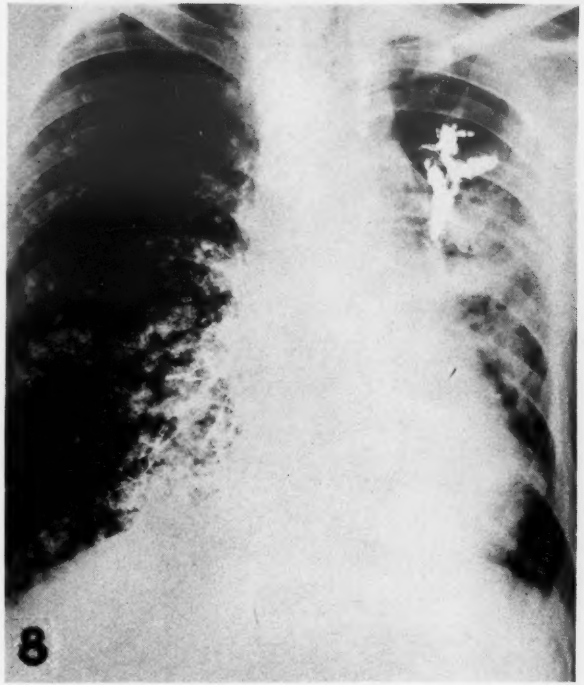


FIG. 8. Bronchogram made in November, 1943, showing bronchiectasis of left upper lobe. Compare with cast shown in Figure 9.

FIG. 9. Bronchovascular cast of left lung removed surgically in January, 1949. There is bronchiectasis of all segments. The greatly enlarged bronchial artery to the lower lobe has been injected with black plastic. In the upper lobe there is retrograde injection of the bronchial arterial plexuses. Close-ups of fields delineated by the white lines are shown in Figures 10 and 11.

FIG. 10. A portion of the pectoral segment of the left upper lobe outlined by the rectangle in Figure 9 is shown in close-up. A bronchial arterial plexus seen as the vermiform mass of intercommunicating vessels about the ectatic bronchus has been injected retrogradely from the pulmonary artery through the large anastomosis (AN).

FIG. 11. Close-up of the portion of the left lower lobe delineated in the lower rectangle in Figure 9. Two large communications of bronchial (BA) and pulmonary (PA) arteries are shown; there are many others of equal or smaller size. Mingling of black and originally red plastic injection media has occurred across the anastomoses (AN). Two of the communicating vessels cross, one above the other, before reaching the branches of the pulmonary artery.

Since the anastomoses in the diseased tissue often involve bronchial and pulmonary arterial vessels of large and almost equal size, and are themselves so large, the possibility of a flow from the bronchial collaterals backwards within the pulmonary arteries towards the hilum suggests itself. It would be difficult to demonstrate such a reverse flow within individual pulmonary segments and subsegments. In the patient with universal bronchiectasis of the left side whose angiocardiagram is illustrated in Figure 7,

TABLE 1

OXYGEN CONTENT AND SATURATION AND BLOOD PRESSURE IN PATIENT WITH BRONCHIECTASIS INVOLVING ALL SEGMENTS OF LEFT LUNG

Source	O_2 *vol. %*	*% sat.*	*Mean pressure*[b]	*Resting pressure*[c]	*Lowest pressure*[d]
Prox. rt. ventricle	12.11	67.5	21.6	54/-1	49/-8
Outflow rt. ventricle	12.04	67.1	21.3	37/12	32/8
Prox. pulm. art.	12.64	70.3	33.3	46/29	42/23
Lt. pulm. art.	16.23	90.3	(36)[b]		44/27
Rt. pul. art.	11.43	63.7	29.4	43/21	34/17
Brachial art.[a]	16.55	92.2			

[a] CO capacity of brachial arterial blood = 17.95 vol. %.
[b] "Mean pressure" = pressure over an entire respiratory cycle obtained by planimetric integration, except for lt. pul. art. where the mean pressure of the lowest pulse curve only could be measured (see Fig. 12).
[c] "Resting pressure" = pressure during interval between respirations; could not be calculated for lt. pul. art. (see Fig. 12).
[d] "Lowest pressure" = pressure of lowest pulse curve, in the inspiratory phase.

however, suggestive evidence was obtained by catheterization of the two pulmonary arteries* (Table 1 and Fig. 12). Blood obtained from the left pulmonary artery had an oxygen saturation close to that of the systemic arterial blood while blood from the right pulmonary artery was venous in character. Since it is possible to suck blood from the pulmonary veins back into the catheter,[11] great care was taken to exert no suction on the syringe but to allow the blood to enter under its own pressure. Pressures were measured with the Hamilton manometer in the two arteries, but a regularly recurrent artifact, presumably due to obstruction of the catheter tip at certain phases of the cycle while in the left pulmonary artery, renders the read-

* Catheterization performed by Dr. Frank D. Gray, Jr. of the Department of Internal Medicine.

ings difficult to interpret. Nevertheless, it is notable that both the systolic and diastolic readings of the lowest pulse curves with the catheter in the left pulmonary artery were higher than the highest with the catheter on the right side. The surgically resected left lung showed universal bronchiectasis

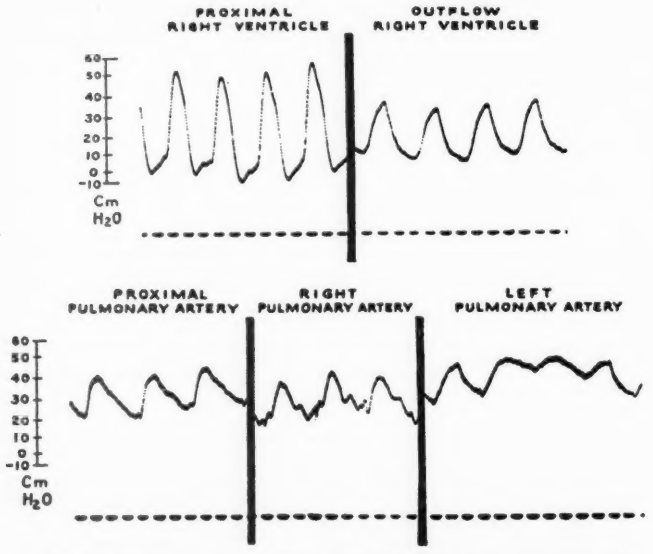


FIG. 12. Pressure curves obtained with a Hamilton manometer in close succession, during catheterization of the patient whose roentgenograms and bronchovascular casts are shown in Figures 6 to 11. There is damping of the pulse wave in the outflow tract of the right ventricle. An artifact recurs regularly during the expiratory phase. For this reason only pressures of the lowest pulse wave during the inspiratory phase are measured and recorded in Table 1.

and innumerable large anastomoses between the bronchial and pulmonary arterial systems (Figs. 9, 10, 11). Further observations of this type are obviously necessary, but patients with massive unilateral bronchiectasis who will submit to the necessary technical procedures are not common. It is important in seeking confirmation to obtain individuals with universal involvement of all segments, for although reverse flow may take place locally, it may not be possible to obtain the evidence in the main pulmonary artery unless all the segments are involved. Attempts are being made in this laboratory to produce unilateral bronchiectasis in dogs by obstructing the

left main bronchus with a timothy grass plug.[5] The condition has developed in some of these animals, but the incidental mortality has been high and we have not as yet been able to obtain a complete study. It is also planned to re-anastomose the distal and proximal ends of the pulmonary artery in dogs who have survived a ligation of this vessel for many months and developed an extensive collateral circulation. Then the circulatory analogue of the massive bronchiectasis should be available for study. The re-anastomosis should be possible since the pulmonary artery remains patent distally despite its ligation.[33]

In the patient with chronic pulmonary disease, the collateral circulation may thus take two courses: the part that enters what remains of the pulmonary capillary bed is transferred to the left auricle, and is thus a burden upon the left heart alone, as in the dog with the ligated pulmonary artery. In contrast with the experimental animal, however, the pulmonary artery in the patient is patent proximally and thus some of the collateral may flow in reverse within the pulmonary arterial system impelled by the higher pressure in the bronchial arteries, to be distributed to normal pulmonary substance where it is added to the output of the right ventricle.

It is clear that the sizable anastomoses between a bronchial and pulmonary artery represent points of increased peripheral resistance within the lung whose significance in producing pulmonary hypertension, as suggested by Wood and Miller,[43] must be evaluated. Blood from the right heart that is shunted away from these points of resistance, together with blood that may actually be flowing backwards through the anastomoses, is directed into the remaining capillaries. The capillary bed of the lung is, however, adaptable to a high degree and even a fraction of it can accommodate an enormously increased flow without pulmonary arterial hypertension. In our own laboratory, the dogs with a ligated pulmonary artery, in whom a single lung obviously received the entire output of the right ventricle, when run at 4 miles per hour on a treadmill have not shown any increase in the right ventricular pressure.* Furthermore, Cournand[8] has recorded observations on patients with patent ductus arteriosus in whom there was a flow from the aorta into the pulmonary artery of as much as 8 liters/min., without pulmonary hypertension. These observations indicate that the points of communication of the pulmonic with the systemic circulation must be very numerous before one may expect them to increase significantly the resistance against which the right ventricle has to work. They will do so when the remaining normal capillary bed has been sufficiently diminished.

* Unpublished experiments.

Of particular interest will be the study by the injection method of emphysematous lungs. We have not as yet encountered in the necropsy series an instance of "pure" emphysema, without more localized fibrous lesions, in which there was cor pulmonale.

Mechanisms concerned in the development of collateral circulation. The exact nature of the stimulus to the development of the collateral circulation is obscure; in some instances several mechanisms may be involved. By reference to William Snow Miller's[39] drawing of the region of the respiratory bronchiole and the tissues beyond, the course of events after ligation of the pulmonary artery may be visualized. It is evident that an elongated meshwork of capillaries derived from the pulmonary artery separates the capillary beds in the terminal alveolated portions of the respiratory tree from the end branches of the bronchial arteries in the walls of the respiratory bronchiole. Peripheral passage of blood entering the latter vessels is resisted by the friction of the capillaries and by counter pressure within the distal capillaries transmitted from the pulmonary artery. When the ligature is applied, this counter pressure is reduced, permitting a further progress of bronchial arterial blood into the capillaries of the alveoli. Having thus available the entire capillary bed, where the peripheral resistance is low, it is not surprising that there should be an increase in blood flow within the bronchial arteries. The exact mechanism responsible for the subsequent enlargement of the latter is not clear. In recent experiments[36] with newborn dogs where the left pulmonary artery was ligated while the right pulmonary artery was constricted to a degree sufficient to produce within three months a very extensive hypertrophy of the right heart with ultimate failure, it was observed that a very extensive collateral developed on the side of the complete ligation, while on the right side there was only a moderate increase in the collateral circulation (Figs. 3-5). This suggests that complete interruption of the pulmonary artery is a much greater stimulus than merely a moderate reduction in the pressure within this vessel. Reduction in the pressure distal to the constriction is suggested by the thin wall and great dilatation of the pulmonary artery—the post-stenotic dilatation that can be produced by similar means in the aorta.[18] There the pressure is known to be much less beyond the ligature.

The existence of a hormonal factor in the genesis of collateral circulation is suggested by the fact that in the growing puppies the collateral on the side of the complete ligation is very much greater than we had observed in a comparable three- to five-month interval in any of a series of adult animals. Whether growth hormones can affect the development of collateral

circulation remains to be tested in control experiments. The converse effect of the adrenocorticotrophic hormone of the pituitary on the formation of granulation tissue, including its capillaries, has received attention recently.

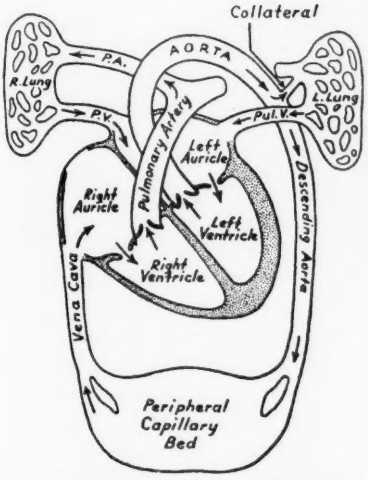


FIG. 13. Schematic representation of the course of the circulation in the dog whose left pulmonary artery has been ligated some months previously. The extensive collateral is represented as a large branch from the aorta. It is obvious that blood entering the left lung from the aorta is returned through the capillary bed of the lung immediately back to the left auricle, pursuing a circle independent of the right heart. The output of the left ventricle thus exceeds that of the right by the volume of the collateral, which is thus a burden only on the left side of the heart.

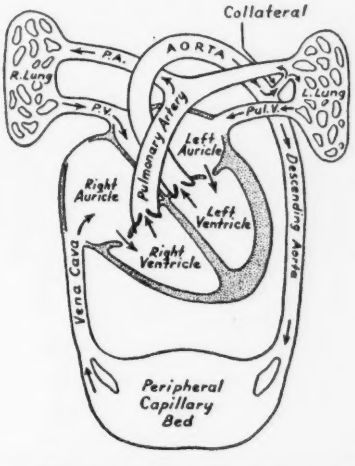


FIG. 14. Schema of the course of the circulation when an extensive collateral circulation has developed in chronic pulmonary disease of the left side (e.g. bronchiectasis involving all segments of the lung as in Figures 9-11). Some of the blood entering the left lung by the collateral channels, represented here as a large branch of aorta, may be distributed through the remaining capillaries of the lung, to pursue the same course as in Figure 13 and with the same physiological connotation. Some blood may, however, enter the pulmonary artery through the anastomoses which exist, impelled by the higher pressure in the collateral channels to flow in reverse, toward the hilum, within the pulmonary artery.

Difficult to visualize also is the process by which the macroscopic stomata develop between the bronchial and pulmonary arteries as the collateral expands, unless it be by gradual dilatation under the stimulus of increased flow in certain originally capillary channels, as during embryogenesis. We have not observed in any of this material, nor in diseased lungs, precapillary

communications with the pulmonary veins; why these should be spared when the arteries are involved is not clear.

In human disease, obstruction of the pulmonary artery or its branches takes place under certain circumstances and thereby, if the experimental dog sets the pattern, contributes to the development of collateral circulation. In tetralogy of Fallot, Rich[24] has demonstrated a high incidence of recent and organizing thrombi in the pulmonary vessels and we have had opportunity to confirm this observation.[10] Thrombotic and endarteritic processes are also frequent in chronic fibrosing conditions of the lung such as tuberculosis and silicosis.[18]

In disease there may be additional demands in the pulmonary substance for oxygenated blood that are met, by mechanisms largely unknown, in the expansion of the collateral circulation. Among them is the support of the elements of the granulation tissue that precedes the fibrosis, the hypertrophied muscle, and excessive lymphoid substance found in the bronchi of many patients with bronchiectasis.[24] Presumably the capillaries in the granulation tissue are derived from both the bronchial and pulmonary arterial systems. Such capillaries may come to join as is common in developing networks of the finest vessels, and then they may enlarge and persist as the anastomoses that have been described; such enlarged vessels have been found in granulation tissue elsewhere.

Summary

In chronic pulmonary disease and in certain forms of congenital heart disease there may develop an extensive collateral circulation and large anastomoses of the bronchial with the pulmonary arteries. Similar changes can be induced in the dog by ligation of the pulmonary artery, and the collateral vessels in the experimental animal come in time to carry very large quantities of blood, equal at the end of 18 months to approximately one-third of the output of the right ventricle. Since this blood from the aorta is returned by the pulmonary veins directly to the left auricle, it is a burden entirely on the left side of the heart whose output then is greater than that of the right by a volume equal to the collateral flow. In human lungs where the pulmonary artery is patent proximally, there may be a division of the collateral stream. The part that passes through the capillaries of the lung is, as in the experimental dog, added to the work of the left heart; but another part impelled by the higher pressure in the bronchial artery may pass via the anastomoses into the pulmonary artery against the direction of the normal stream, toward the hilum of the lung to be directed

together with the output of the right heart into undamaged parenchyma. Shunting of blood away from diseased pulmonary tissue is suggested in angiographic observations, and by the absence of desaturation despite involvement of as much pulmonary substance as that of an entire lung. The anastomoses between the bronchial and pulmonary arteries represent points of increased resistance that, when sufficiently numerous, will increase the work of the right ventricle.

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VASCULAR LESIONS IN THE DOG FOLLOWING THYROIDECTORY AND VIOSTEROL FEEDING*

WILLIAM B. McALLISTER, JR., AND LEVIN L. WATERS**

Introduction

For over twenty years it has been known that overdosage of viosterol produces widespread arterial lesions of a chronic type in the experimental animal, particularly in the thyroidectomized dog.[4, 7] Investigators have described calcification of the media of the aorta as the principal change produced,[5, 10, 12, 18] and many have observed in these same animals a fragmentation or marked distortion of the internal elastic lamella accompanied by the deposition, on or in it, of more or less calcium. This latter change was not so constant nor so widespread as was that in the aorta and usually affected only its larger musculo-elastic branches. Only rarely were proliferative intimal lesions described.[1, 2, 6]

Our interest in these lesions was stimulated by the work of Steiner and Kendall[17] who reported having produced arteriosclerosis in dogs fed thiouracil and cholesterol for seventeen to twenty months, and also by a report of Dr. John Peters[14] of a study of a number of hyperthyroid patients who had received long courses of treatment with thiourea. In some of these individuals widespread acute arterial necroses were found at autopsy. Although allergy to the thiourea and iodine was of course suggested as an explanation for these lesions, it was also suggested that the hypothyroid state resulting from the prolonged treatment with thiourea might have had something to do with their production.

The working hypothesis for the present investigation was that there might be antecedent acute arterial lesions in viosterol-fed dogs, particularly if thyroidectomized, similar to those observed clinically by Dr. Peters, and also that there might be a relationship between these acute lesions and the usually described chronic medial calcification. Further, the changed metabolism as expressed by the altered lipid pattern of the blood might alter their morphogenesis.

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Methods

Twenty mongrel dogs of both sexes were thyroidectomized. Two or more parathyroid glands were preserved with extreme care, and the animals were not given viosterol until at least a week later. If tetany developed, the dog was sacrificed and discarded. Commercial viosterol* in doses averaging 50,000 units per kilogram of body weight was added daily to a diet consisting of about a pound and a half of pig kidney. Kidney was fed as the cheapest form of lean meat available in quantity, and it had no special significance in the experiment. Animals which were thyroidectomized but did not receive viosterol, and normal animals fed the same amounts of viosterol served as controls.

Free and total cholesterol levels were determined by the method of Schoenheimer and Sperry,[10] phospholipid phosphorus by a modified Youngburg method,[9] serum calcium by the method of Roe and Kahn,[15] serum inorganic phosphorus by the method of Lowry and Lopez,[11] and blood nonprotein nitrogen by micro-Kjeldahl digestion and steam distillation of the ammonia formed. Blood for these determinations was collected under oil (after the animals had been fasted for 24 hours) before, and one week after, thyroidectomy and at frequent intervals after viosterol feeding was instituted.

Results

No gross vascular changes were observed during the first 10 to 14 days of viosterol feeding, but after that arterial lesions were regularly produced by this regimen. The earliest lesions were focal and rather widely scattered, later they were more widespread and tended to become confluent. In fact, the changes were more widespread than was anticipated from the literature, and with time, there developed a definite distributional pattern not described by others, see Table 1.

Aorta. During the first 10 days of viosterol feeding following thyroidectomy, only a marked edema of the inner third of the media is seen histologically. This is a constant finding in the aortas of the viosterol-treated animals. Even months later it is prominent in those portions of the vessel which are apparently normal on gross examination.

After two or more weeks on the viosterol regimen, focal necrosis of the inner half of the media of the aorta becomes increasingly common, particularly at its root and in its thoracic portion. The elastic fibers in the center of the lesions are destroyed or fragmented, and there is a surrounding acute, purulent, inflammatory reaction (Figs. 1 and 2). The wall of the vessel adjacent to such a purulent focus is extremely edematous, causing considerable distortion of the normal elastic fiber pattern. Intimal proliferation (Fig. 3) becomes notable only after the necrotic center of the lesion

* Kindly furnished by the International Vitamin Division of the Ives-Cameron Co., Inc., Brooklyn, N. Y. Viosterol in oil, 1 gram = 10,000 units vitamin D, *U.S.P.*

begins to liquefy. Concurrently, new capillaries which invade the outer media are formed by budding of endothelial cells at the medio-adventitial junction (Fig. 4). Lipid stains (Sudan IV, Sudan black, and treatment with osmic acid) demonstrate that stainable material is found scattered about the necrotic mass in the media and in the zone of reaction in the adventitia, distributed in the form of fine extracellular droplets. Weeks or months later salts, predominantly calcium, are found to have been deposited

TABLE 1

DISTRIBUTION OF VASCULAR LESIONS IN VIOSTEROL FED DOGS

									Coronary arteries			*Peripheral arteries*				
	Number of dogs	Root of aorta	Thoracic aorta	Abdominal aorta	Pulmonary artery	Left auricular endocardium	Thrombi and location		Acute panarteritis	Intimal proliferation	Foam cells	Carotid	Subclavian	Iliac and femoral	Mesenteric	Renal
THYROIDECTOMIZED DOGS																
Fed viosterol 4 weeks or less	8	6	4	1	7	6	2	l. auricle	1	4	0	3	4	5	0	0
Fed viosterol 4-10 weeks	6	6	5	4	6	5	1	pul. artery	0	5	1	4	5	6	1	1
Fed viosterol 10 or more weeks	6	6	5	5	5	4	1	aorta organized	0	6	4	4	4	5	2	2
Totals	20	18	14	10	18	15	4		1	15	5	11	13	16	3	3
NORMAL DOGS .	8	4	2	1	6	2	0		0	3	0	2	3	5	0	0

in such lesions (Fig. 5), and there may be no evidence of an inflammatory reaction or of intimal thickening. Under these conditions, stainable lipid is also lacking. However, a subacute intimal inflammatory process may be superimposed on such an apparently healed medial lesion (Fig. 6). When such is the case, fine extracellular droplets of lipid are easily demonstrated. A small organizing mural thrombus was attached to the newly proliferated intima of one such lesion.

Figures 7 and 8 are photographs of the aortas of two thyroidectomized animals fed viosterol for 23 and 17 days respectively. The similarity in dis-

tribution between these lesions and those of early human arteriosclerosis in the aorta is apparent.

Pulmonary artery. In the main pulmonary artery the earliest and most severe lesions are found in that portion which crosses over the aorta. The changes are similar in every respect to those described in the aorta with the exception that hemorrhage associated with the acute inflammatory reaction is a more frequent finding (Figs. 9 and 10). After several weeks of viosterol treatment the whole circumference of the pulmonary artery is involved. The vessel becomes dilated, and the intima roughened by many raised irregular plaques. No lesions are observed in the smaller intrapulmonary branches.

Left auricle. After three or more weeks of viosterol feeding, lesions of that portion of the left auricular endocardium extending upward from the base of the anterior leaflet of the mitral valve were found in 15 of the thyroidectomized dogs. As in the aorta, the earliest change is an acute inflammatory reaction in the subendocardial tissue. At first, such lesions are hemorrhagic (Fig. 11), and in three animals organizing thrombi were attached to the roughened endocardium (Figs. 11 and 12). With more prolonged treatment with viosterol, calcium salts are deposited in the subendocardial tissue, so that whitish linear streaks run perpendicular to the attachment of the mitral leaflet and upward toward the right pulmonary vein. Scattered but similar lesions are also found above the posterior mitral leaflet (Fig. 11), but the characteristic site is that described.

Coronary arteries. Acute panarteritis, characterized by focal fibrinoid necrosis of the arterial media, local destruction of the elastic fibers of the internal elastic lamella, and marked intimal and adventitial proliferation was observed in one thyroidectomized dog fed viosterol for three weeks (Figs. 13 and 14). However, the changes usually observed after this period of treatment are much less severe. There is edema of the muscular wall of the main coronary arteries and of their larger subepicardial branches with distortion and fragmentation of the internal elastic lamella. Concomitantly, fibrous tissue is laid down in the zone of damage forming an elevated intimal plaque. An advanced lesion of this type is shown in Figure 15. Lipid stains of such a vessel are negative.

With more prolonged (10-20 weeks) viosterol treatment after thyroidectomy, plaques are found regularly in the larger coronary arteries. However, lipid-containing foam cells histologically identical with those seen in early human arteriosclerosis are now present in some of the lesions. Usually the foam cells lie between the intimal lining of the vessel and the internal

elastic lamella (Fig. 16), but if that structure has been fractured, they also infiltrate between the muscle cells of the arterial media beneath the break. (Figs. 17 and 18). The lipid in these cells is contained in large droplets which are osmophilic and sudanophilic and which are birefringent under crossed Nicol prisms. If a frozen section of a plaque containing foam cells is heated gently, and light pressure applied to the coverslip, droplets are released which show characteristic Maltese crosses under polarized light. When there are many foam cells in the intimal plaques of the coronary arteries, very few, if any, are present in the other vascular lesions and the cells of the reticuloendothelial system are normal.

One cannot predict with certainty from blood lipid levels whether foam cells will be present in the plaques in the coronary arteries. For example, Figure 16 shows the coronary artery of a thyroidectomized dog whose total cholesterol level averaged only 230 mgm.% during the 10 weeks it was fed viosterol. Conversely, in another thyroidectomized dog with persistent blood cholesterol levels three times as high, only fibrous plaques were found after 13 weeks of viosterol treatment. The levels of the other lipids were comparable in the two animals. There is, however, a definite correlation between the presence of foam cells and the level of blood lipids in most cases.

Other peripheral arteries. Dilatation and medial calcification, often segmental, like that seen in human disease of the Monckeberg type, are regularly produced in the iliac, femoral, subclavian, and carotid arteries of the thyroidectomized dog fed viosterol (Fig. 7). Beneath a greatly distorted and elongated internal elastic lamella, groups of smooth muscle cells of the arterial media are seen to be calcified without other apparent histological change. Rarely, small fibrous intimal plaques are formed when dilatation of the vessel causes a portion of one of these calcified foci to project into the lumen. Similarly, calcification of the internal elastic lamella was observed histologically in the large intrarenal and mesenteric arteries of three animals. Renal arteriolosclerosis was not produced on this regimen, but focal calcification of the basement membrane of the secretory tubular epithelium, atrophy, and calcification of the epithelial cells were regularly observed histologically. Numerous calcified casts were also present in the collecting tubules.

Metabolic effects. Much experimental work has been done to determine the toxic effects of excessive doses of viosterol upon laboratory animals. In the normal dog there is produced a marked metabolic disturbance characterized by hypercalcemia and hyperphosphatemia, and by elevation of all the blood lipid levels. In addition, the blood nonprotein nitrogen rises, and

there is hemoconcentration. These alterations are marked at the end of only one week of viosterol treatment, and although certain adjustments take place with time, they become even more pronounced after one month, Table 2.

TABLE 2

BLOOD FINDINGS IN FOUR REPRESENTATIVE EXPERIMENTAL DOGS

	Free cholesterol	Total cholesterol	Phospholipid phosphorus	Fatty acid titer mEq./L.	Serum calcium	Serum inorg. phosphorus	N. P. N.	Hematocrit
Dog B108 Normal Control fed viosterol.								
Control period	46	116	11.5	11.4	12.0	4.8	28.2	38
Seven days after viosterol .. 50,000 units U.S.P. per kg.	44	96	14.3	15.0	13.8	4.9	53	39.2
21 days after viosterol	134	300	22.5	21.6	21.8	8.1	86	53.2
60 days after viosterol	176	420	22.1	17.6	17.8	7.2	74	51
70 days after viosterol	200	390	21.0	20	22.6	5.6	54	46
Dog B89 Thyroidectomized.								
Control period	67.5	120	12	13.8	11.5	5.0	30	
Seven days after thyroidectomy	83.0	144	18.6	19	11.6	5.6	35	
Seven days after viosterol ..	167.0	410	27.6	28.8	19.6	6.6	70.7	
17 days after viosterol	137.0	276	24.5	28.4	27.9	6.6	105	
Dog B97 Thyroidectomized.								
Control period	48	70	17.6	17.2	13.7	5.3	35.0	48
Seven days after thyroidectomy	55	110	19.3	18.8	13.8	5.0	44.8	49
Six days after viosterol	167	548	32.8	34.4	15.9	7.1	46.9	48
30 days after viosterol	411	688	36.7	38.8	20.2	5.9	46.2	48
60 days after viosterol	250	648	32.0	40.4	14.7	4.4	35.9	39
90 days after viosterol	222	480	30.0	31.6	16.5	6.2	53.2	43
120 days after viosterol	264	840	37.0	38.0	14.0	6.1	56.0	46
140 days after viosterol	397	872	35.2	44.0	24.6	5.4	34.3	50
Dog B86 Thyroidectomized Control.								
Control period	35.0	100	15.1	12.0	15.0	6.2		40
Seven days after thyroidectomy	57.0	128	14.6	18.4		7.8	30.8	37
60 days after thyroidectomy .	78.0	196	17.5	17.6	12.6	7.8	31.5	36
120 days after thyroidectomy	76.5	220	17.7	15.6	12.2	7.4	28.7	35

All the metabolic changes are more profound when the dog is thyroidectomized before viosterol overdosage. As will be seen from Table 2, however, the greatest effect of thyroidectomy is upon the lipid metabolism. Blood cholesterol levels were always doubled, and often increased four-fold or more. Similarly, serum levels of phospholipid phosphorus and the fatty acid titre were at least doubled. Thyroidectomy alone produces no such profound metabolic effects (Table 2), and the hematocrit and nonprotein nitrogen levels remain within normal limits. However, both of these are persistently elevated when the dog is given toxic doses of viosterol. What may be the effect of the meat diet and the hemoconcentration upon the blood level of nonprotein nitrogenous material cannot be determined from these experiments.

Arterial blood pressure was studied in 10 of the animals. In none was there a real hypertension. One normal animal fed viosterol for 10 weeks had a mean systolic pressure of 160 mm. of mercury at the end of the experiment.

Control animals. Eight normal dogs were fed on the same viosterol schedule as were those which were thyroidectomized. Vascular lesions having the same distribution as those in the thyroidectomized dogs resulted, but in every instance they were fewer, less acute, and less extensive. Moreover, the period of feeding of the toxic doses of viosterol had to be extended considerably as there were no grossly obvious lesions until the dog had been under treatment for six weeks or more. No foam cells were observed in the vessels of two animals treated for 70 days.

No vascular lesions occurred in 120 days in those dogs which were thyroidectomized but not fed viosterol.

Discussion

Since the work of Goldblatt,[3] it has been known that in the dog persistent hypertension when associated with renal failure is followed by focal, acute necroses in the systemic arterioles. Acute lesions of large vessels, including particularly the pulmonary artery and aorta have been observed in this laboratory in association with experimental renal ischemia.[10] Furthermore, the work of Holman,[9] involving severe renal damage and the feeding of special diets containing a high percentage of lipid, disclosed lesions having the same morphology and distribution as those described in this communication. It is noteworthy that there was in our experimental animals some

evidence of azotemia. This may have had a role in the production of the vascular changes described.

Other investigators who have studied the toxic effects of viosterol upon the experimental animal have used different routes for administration of the vitamin. They have given it in single or multiple doses over varying periods of time, and different laboratory animals have been used. It is difficult, therefore, to draw conclusions between their results and ours. However, certain factors are worthy of comment. Ham and Ham and Lewis[5, 6] report that in rats following a single massive parenteral dose of viosterol, calcification of the muscle fibers of the arterial media occurs without previous anatomical change having taken place in the fibres. With this, Duguid,[2] who also studied the changes in rats, disagrees. He believes, on the basis of histological evidence, that the primary lesion is a degeneration of the arterial smooth muscle, and that calcification follows this change. Both of these authors agree that the intimal reaction is secondary to the medial lesion.

Under the conditions of the present experiments, one of two types of change always preceded the more chronic vascular lesions. The first of these was medial necrosis accompanied by an acute inflammatory reaction. The other was fracture, disruption, or other histologically obvious distortion of the internal elastic lamella of the smaller arteries, or of the elastic fibers in the larger arteries or in the subendocardial tissues of the left auricle. If neither of these changes was present, edema of the media, almost certainly a reversible process, was the only alteration observed, and none of the later changes was produced. Intimal plaques may or may not form in response to the medial lesions (Figs. 5 and 6), but intimal proliferation was not observed in these experiments without underlying medial change.

The role of the thyroid is difficult to assess. Vascular lesions did not follow thyroidectomy alone, but they were produced more quickly and were greater in extent in those dogs whose metabolism was already altered by thyroidectomy. Moreover, lipid-containing foam cells were not observed, even with prolonged viosterol treatment, in the coronary artery lesions of the normal dogs. Menne, Beeman, and Labby[13] observed that there was greater lipophage deposition in the aortas of thyroidectomized rabbits fed cholesterol than in normal or thyroid-treated animals kept on the same diet for the same period of time. However, it must be emphasized that arteriosclerosis is a focal disease, and that local as well as metabolic factors must therefore be sought to explain the focal involvement of the vessel wall. In

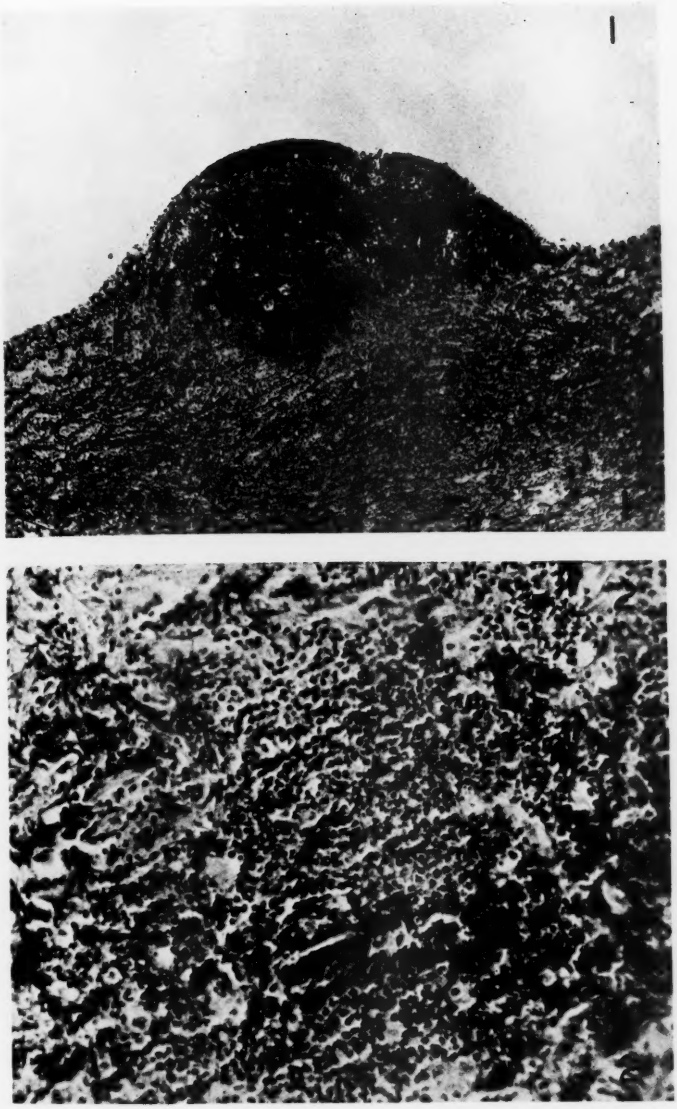
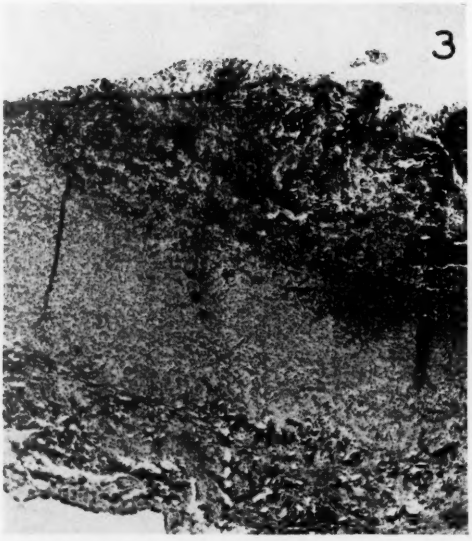


FIG. 1. Focal necrosis with acute inflammatory reaction in inner third of media of aorta. Thyroidectomized Dog B89 fed viosterol for 17 days. x 52. Note edema of wall of aorta.

FIG. 2. Necrosis and purulent exudate in center of aortic lesion shown in Fig. 1. Dog B89. x 350.



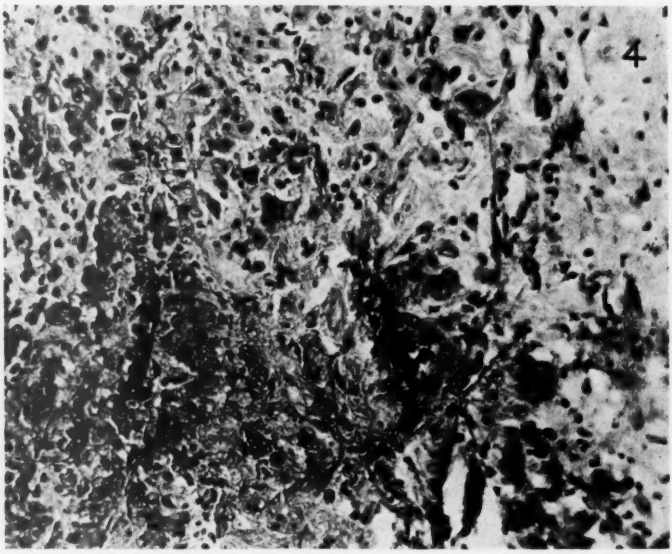
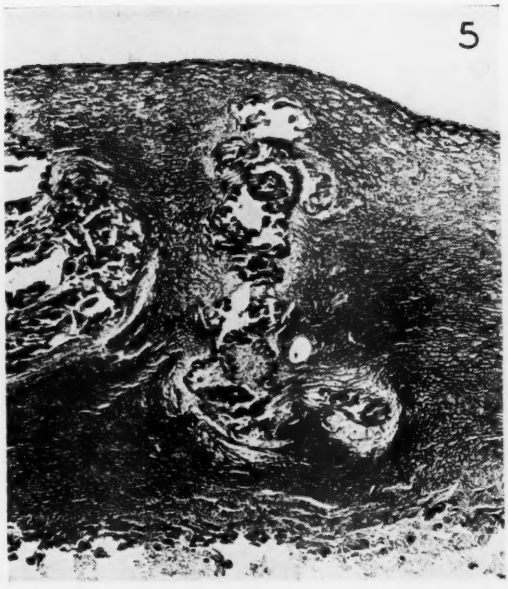


FIG. 3. Larger necrotic focus in aorta of Dog B89 with beginning liquefaction in central portion of inflammatory lesion. There is early intimal proliferation and capillary buds are forming at junction of the media and adventitia. x 40.

FIG. 4. Endothelial cell knots and capillary buds invading media of aorta from adventitia. Dog B89. x 335.



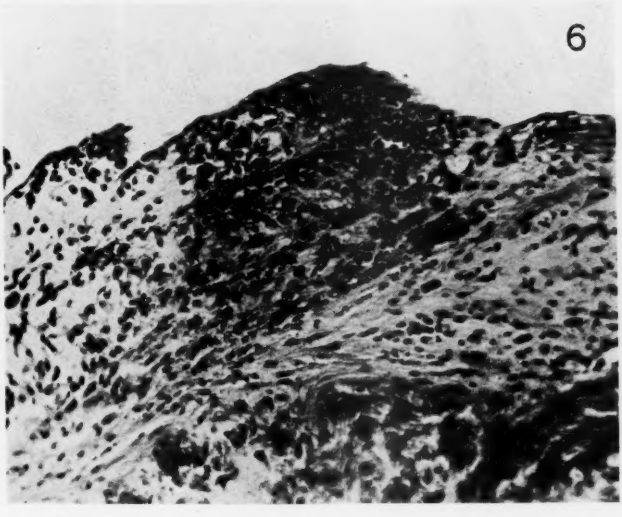


FIG. 5. Healed "calcified" aortic lesions in a thyroidectomized dog after 75 days of viosterol feeding. Note lack of intimal reaction. Dog B106. x 37.

FIG. 6. Acute and subacute intimal reaction in aorta of thyroidectomized dog fed viosterol for 23 days. An older, "calcified" lesion is seen in the lower half of the photograph. Dog B90. x 320.

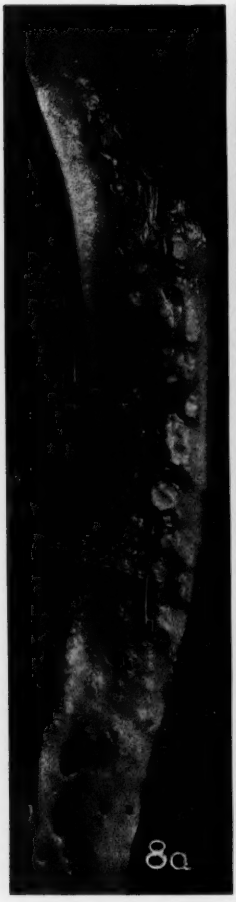


FIG. 7. Aorta of thyroidectomized Dog B90 fed viosterol for 23 days. There are many elevated plaques in both thoracic and abdominal segments. See also Figs. 6 and 12.

FIG. 8A. Thoracic portion of aorta of Dog B89 (see also Figs. 1, 2, 3, 4, and 11). Note similarity of distribution of these acute inflammatory lesions to that of early human arteriosclerosis of the aorta. Actual size.

FIG. 8B. Aorta of Dog B89. Photograph made at same scale as Fig. 7 for comparison.

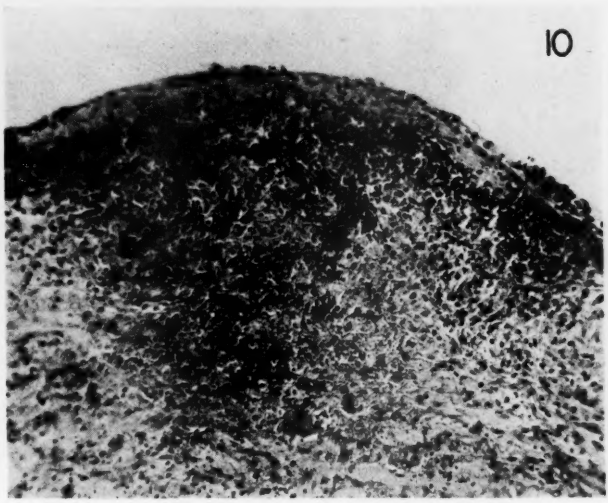


FIG. 9. Main pulmonary artery of thyroidectomized Dog B93 after 12 days of viosterol feeding. There are acute inflammatory foci in the inner half of the edematous media. x 38.

FIG. 10. Higher magnification of inner half of same pulmonary artery. Early intimal proliferation overlying an acute inflammatory focus. B93. x 220.

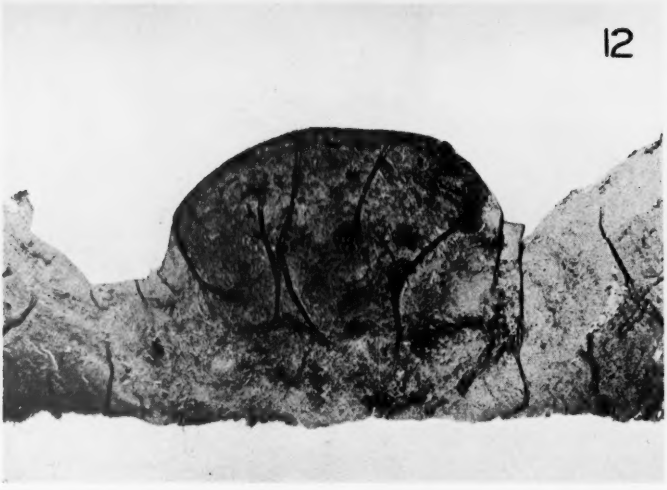
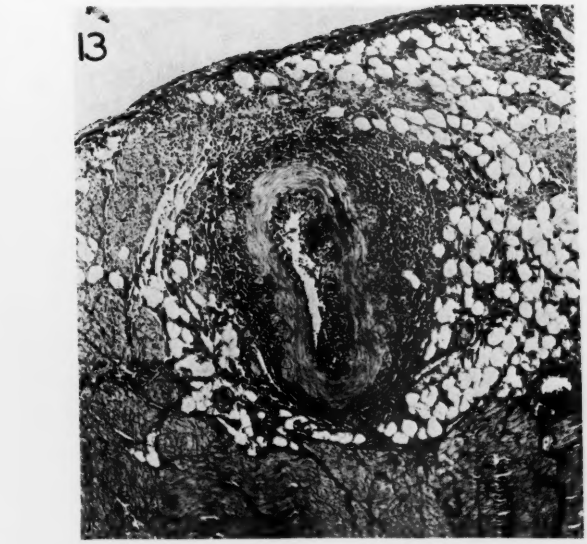


FIG. 11. Left auricular endocardium of Dog. B89. There is an acute, hemorrhagic inflammatory reaction in the subendocardial connective tissue above the base of the anterior mitral leaflet. A ball thrombus is attached to a similar lesion above the posterior leaflet. Note (arrow) the thickened coronary artery.

FIG. 12. Organizing thrombus attached to left auricular endocardium of Dog B90. Note the subendocardial inflammatory reaction. x 17.



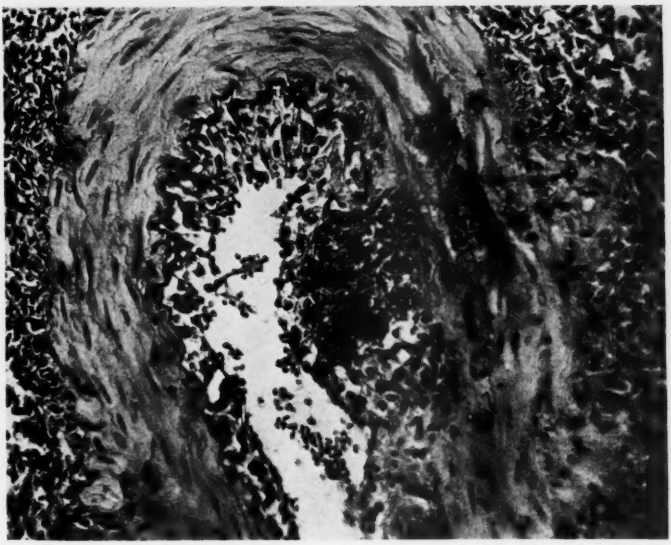


FIG. 13. Left anterior descending coronary artery of Dog 319, x 57. There is an acute panarteritis with marked intimal and adventitial proliferation and focal necrosis of media. Viosterol feeding for 21 days after thyroidectomy.

FIG. 14. High power view of same coronary artery to show necrosis of media and disruption of internal elastic lamina. x 340.

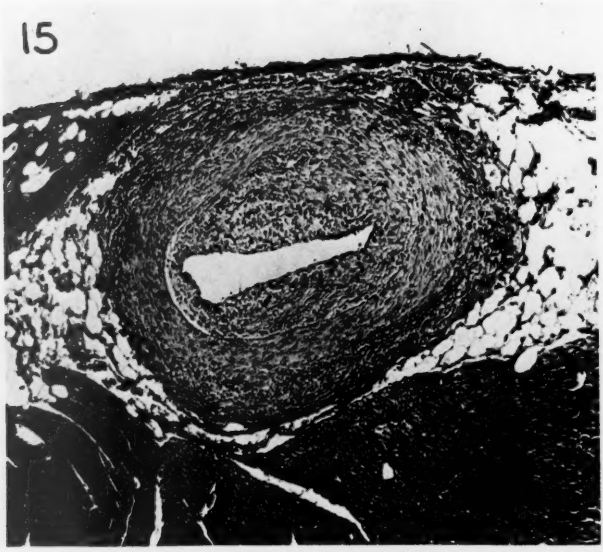
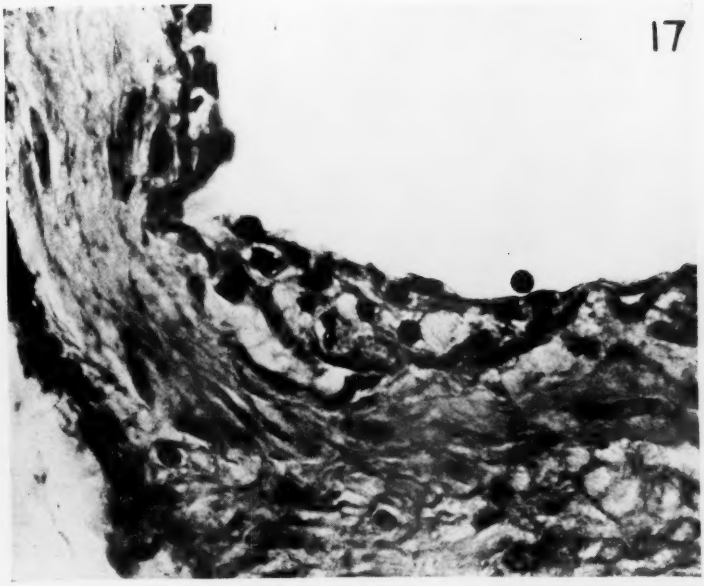


FIG. 15. Marked intimal fibrosis of left main coronary artery. Thyroidectomized Dog B71. Viosterol feeding for 30 days. x 70.

FIG. 16. Subintimal lipid filled foam cells in coronary artery of Dog B59. x 50, Sudan Black stain. Thyroidectomized and fed viosterol for 70 days.



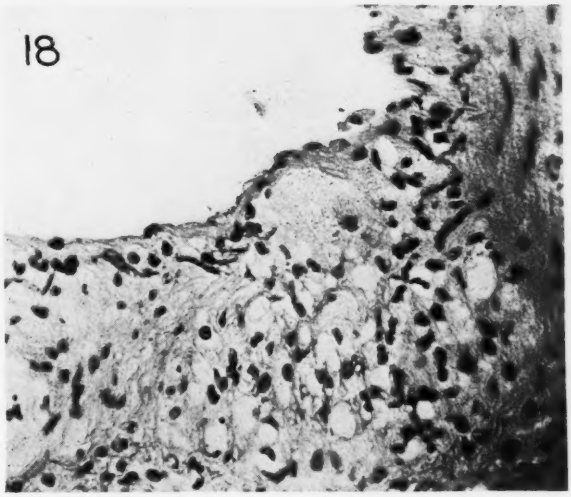


FIG. 17. A small intimal plaque from a coronary artery of Dog B105 thyroidectomized and fed viosterol for 75 days. Most of the foam cells lie between the internal elastic lamella and the endothelium. Note that two or three foam cells have begun to spread out beneath the elastic lamina through a break in its continuity. There is edema of the arterial wall beneath the lesion. x 850.

FIG. 18. Intimal plaque containing larger foam cells from coronary artery of thyroidectomized Dog B103 fed viosterol for 92 days. x 425.

this connection, it is interesting that fatty change did not occur generally in the cells of the reticuloendothelial system of the experimental animals.

Summary

Acute arterial and endocardial lesions are produced by feeding toxic quantities of viosterol to thyroidectomized dogs. Their distribution and pathogenesis are described.

These lesions are anatomically similar to those occurring in dogs with experimental renal disease. Their distribution in the aorta corresponds to that of early human arteriosclerosis.

Especially after prolonged hyperlipemia, lipid is deposited in the intima of the affected coronary arteries selectively at the sites of lesions.

Finally, a practical experimental method is described for producing in dogs arterial lesions that allow the study of the deposition and transport of lipid. This can be approached from the standpoint of the morphology of the vessel wall and by the physical and chemical composition of the blood.

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THE ISOLATION OF BRUCELLA ORGANISMS FROM APPARENTLY HEALTHY INDIVIDUALS*

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Although the etiological agent of brucellosis has been known for many years, this widespread infection remains one of the greatest enigmas in modern medicine.

Perhaps the principal source of confusion is the difficulty of establishing a definite diagnosis of brucellosis. This is particularly true of the chronic form of this disease. While the history, clinical picture, laboratory procedures, intradermal tests, and pathological findings are of great assistance, a positive diagnosis usually can be made only by demonstrating the brucella organisms. These bacteria are quite difficult to culture, being very fastidious in their growth requirements and demanding a prolonged period of culture. If the tissue or blood being cultured is contaminated, or if aseptic technique is not carefully maintained in the collection of the material, the more slowly growing brucella may be overgrown by other organisms. It is essential that the proper carbon dioxide tension be maintained as the brucella organisms may be of the abortus species. Even in acute cases having chills, the number of brucella in the blood at a given time may be quite limited. Positive identification of the organisms requires specific antigen-antibody reaction.

Even if brucella organisms are cultured from the tissues of a patient, does this prove that the patient has active brucellosis? Perhaps he may only be harboring the organisms in his body—in other words, he may be a brucella carrier. The distinction between a carrier and a person with active disease may be quite difficult to make; indeed, in chronic brucella infections this might be extremely difficult if not impossible. In recent years brucella have been implicated in such conditions as Hodgkin's disease,[25] duodenal ulcer,[5] psychoneurosis,[8] neurasthenia,[14] multiple sclerosis,[2] allergy,[8] subacute bacterial endocarditis,[17] cirrhosis of the liver,[29] and hepatitis.[8] Finding

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brucella organisms in patients suffering from these conditions has led some to consider them to be the etiological agents in these diseases. The most interesting possibility of this type resulted from the discovery of brucella organisms in a case of Hodgkin's disease in 1939 by Parsons and Poston.[25] Following this, several similar cases were reported. In these cases the clinical and pathological findings substantiated the diagnosis of Hodgkin's disease. Forbus and Gunter[10] reported five such cases. Negative agglutinations for brucella were obtained in all of these patients. The organisms obtained at autopsy in four of these cases produced the characteristic lesions of brucellosis upon being injected into guinea pigs; however, the virulence of these organisms was low. Additional support for the possible relationship of brucella organisms to Hodgkin's disease was furnished by the fact that the morbid anatomy of brucellosis and Hodgkin's disease is at times very similar.

In addition to Hodgkin's disease, brucella organisms have been isolated in a number of other conditions. For example, Mettier and Kerr[22] reported a case of acute cholecystitis in which cultures from the gall bladder wall after cholecystectomy yielded brucella. White[30] also obtained these organisms from pus aspirated from the gall bladder. Leavell and Amoss[18] recovered brucella by duodenal drainage from a patient with chronic brucellosis. Simpson[30] reported twelve appendectomies and two cholecystectomies which were performed on patients with undulant fever. The pathological examination of these specimens revealed no evidence of an active inflammatory reaction.

Rossmiller and Ensign[27] have reported hepatitis associated with brucellosis. Necropsy material as described by Hughes[16] and liver specimens obtained by needle biopsies[29] indicate pathological changes in the liver caused by brucella. A possible relationship to cirrhosis has been suggested.

The pulmonary manifestations of brucellosis have been reviewed by Haden and Kyger[12] who state that hilar and peribronchial infiltration, pleural effusion, pleural adhesions and pleural granulomata occur.

De La Chapelle,[17] Moore and Carpenter,[23] and Spink[32] have reported cases of subacute bacterial endocarditis due to brucella.

Woods[37] described five cases showing the classic picture of nummular infiltrates in the cornea associated with serologic or allergic evidence of brucellosis. He pointed out that similar lesions can be produced by the proper inoculation of experimental animals with brucella.

Carryer and Prickman[5] found that duodenal ulcer occurred approximately two and one-half times as frequently among patients who had been infected with brucella as among noninfected individuals. While the numbers involved are too small to be statistically significant, these authors believe that ulcer patients should take greater precautions than usual to avoid contact with brucella organisms.

Brucella were suggested as an etiological factor in intermittent hydrarthrosis by Baker.[1] Hardy[13] and Simpson[30] showed the similarity between the arthritis of rheumatic

fever and brucellosis. According to Hardy,[13] an eroding suppurative arthritis can be caused by brucella.

Sprunt and McBryde[34] called attention to relationship of brucellosis and anemia and reviewed the morbid anatomy of brucellosis.

Griggs[11] pointed out that chronic brucellosis with its multitude of vague signs and symptoms, long duration, recurrences, varied diagnoses and unsuccessful treatment frequently leads to the conclusion that the patient is "neurotic" or that the complaints are functional. A relationship to neurocirculatory asthenia or psychoneurosis has also been suggested.

In this laboratory we have been able to culture brucella organisms from patients with Hodgkin's disease as well as other abnormal conditions. This, together with the above considerations, lead us to seek an explanation other than an etiological one. An alternate possibility seemed to be that brucella organisms had settled out in damaged tissue. Perhaps they were living innocuously in tissues damaged by other agents.

The settling out of organisms in damaged tissue has been recognized for many years. Topley and Wilson[35] state that the localization of bacteria is to some extent determined by their portal of entry into the body but that fundamentally it is a question of the metabolic properties of the organism concerned and the health of the varied organs of the host. This observation is supported by the work of Phemister[20] on the occurrence of infection in damaged bone and by the study of Mallory[21] and his associates on the production of experimental pyelonephritis in rabbits. Lepper[19] reported that transitory obstruction and damage to the ureter is of importance in localizing infective lesions. Lucas[20] attempted to explain the increased susceptibility of damaged renal tissue to hematogeneous infection on the basis of a slower blood flow which allowed circulating bacteria to gain a foothold with resultant necrosis of the walls of the vessels and infection of the surrounding tissue.

The work of Theobald Smith,[31] Buddingh and Womack,[4] Dickey and Forbus,[9] and that of Castaneda,[7] has shown that brucella are able to live and multiply in cells such as macrophages, fibroblasts, reticuloendothelial cells of the spleen, parenchymatous cells of the kidney, and the interstitial cells of the testis. Huddleson[15] pointed out that localization is characteristic of brucella infections in animals.

In an attempt to gain some knowledge as to the frequency of infection by brucella organisms in the Memphis area, 100 patients were selected indiscriminately on the Medical Service of the John Gaston Hospital and were subjected to an intradermal test using Brucellergen. In 39 instances, a positive reaction was obtained. Five of these patients gave strongly positive

reactions and two of them were found to have acute brucellosis. Since Memphis is located within an area in which brucellosis is endemic, it was thought that the hypothesis advanced above could be adequately evaluated by the bacteriological study of diseased tissue. The results of this investigation are reported below.

Methods and materials

Selection of tissue for study. Tissue available for study fell into two categories—surgical specimens and necropsy material. It was believed that with proper methods of handling, tissues removed at operation might be obtained in a less contaminated condition than those from autopsies. Criteria for the selection of tissues for investigation were as follows: (1) that they be available in sufficient quantity to permit adequate bacteriological and pathological examination, and (2) that they be the site of chronic inflammation. Consideration of the surgical pathology reports for 1946 revealed that fallopian tubes and prostatic tissue most often met these requirements. Therefore, after consultation with the Gynecological and Genito-urinary Departments of the University of Tennessee and the John Gaston Hospital, it was decided to use these tissues for study. It was also believed that necropsy material such as lung, spleen, liver, and lymph nodes showing gross evidence of chronic inflammation should be investigated as possible foci of chronic brucella infection.

Method of obtaining tissue to be studied. In a transurethral resection, the first pieces of tissue brought out in the antiseptic irrigating fluid were dropped into a sterile towel and were then placed into an autoclaved container. The remainder of the specimen was sent to the laboratory in the usual manner. When a suprapubic resection was performed, the entire specimen was placed into a sterile container by the surgeon; when a hysterectomy was performed, he placed the tube and ovary into an autoclaved container. Only adult necropsies performed within one hour following death were used for study. Sections of liver, spleen, mesenteric lymph nodes, and lung were removed in a sterile manner.

Methods of culturing tissue. All surgical specimens were cultured within two hours following their removal from the body. After having been cut into smaller pieces with scissors, each specimen was washed through serial beakers containing physiological saline solution; then, the pieces of tissue were transferred to a mortar and a small amount of physiological saline solution was added. The tissue was thoroughly ground, and the suspension was transferred to a tube containing fresh tryptose broth. The tryptose broth culture was then incubated at 37.5° C. in 10 per cent carbon dioxide for 10 days. On the tenth day, a loop of the broth was transferred to a freshly prepared tryptose broth culture and the plates were reincubated for 10 days at 37.5° C. in 10 per cent carbon dioxide.

At the conclusion of this 10-day period, the plates were examined. Any suspicious colonies were studied by gram-stained smears. If a gram-negative cocco-bacillus was noted, it was transferred to sugars and a colony was transplanted to new plates. If there was no evidence of the production of acid or gas in the sugars, the specificity of the organism was determined by agglutination with polyvalent brucella antiserum.

If there were no suspicious colonies, the plates were discarded. Again, a loop of the

tryptose broth culture was transplanted to plates as outlined above, and both the tryptose broth and the plates were incubated an additional 10 days. At the conclusion of this period, the plates were examined as before. Inasmuch as the Brucella species differ from one another in their ability to grow on Bacto-Tryptose agar containing thionin or basic fuchsin, this factor was used to identify the species. Br. melitensis does not require 10 per cent carbon dioxide for growth and will grow in the presence of 1:50,000 thionin and also in the presence of 1:25,000 basic fuchsin; Br. abortus does require 10 per cent carbon dioxide for growth and will not grow in the presence of thionin in a concentration of 1:50,000, though it will grow in the presence of 1:25,000 basic fuchsin. It should be noted that after prolonged artificial cultivation, certain strains of Br. abortus do not require 10 per cent carbon dioxide for growth. Br. suis does not require 10 per cent carbon dioxide for growth and will grow in the presence of 1:50,000 thionin but not in the presence of 1:25,000 basic fuchsin.

Intradermal tests. Intradermal tests, using three antigens and a control simultaneously, were carried out. The antigens employed and their dosage are listed below. Purified protein obtained from Morales-Otero[24] of San Juan, Puerto Rico, was used. This antigen is produced by a modification of Seibert's method for preparing purified protein from tuberculin. The second antigen consisted of the polysaccharide fraction[26] developed from encapsulated brucella at the Lederle Laboratories in Pearl River, New York. The third antigen was Brucellergen,[15] the brucella protein nucleate developed by Huddleson and distributed commercially by Sharpe and Dohme. The control consisted of distilled water.

The three antigens and the control were injected into the volar surfaces of both forearms with a 25-gauge needle employing an inoculum of 0.1 cc. The reactions were measured at 24 and 48 hours. The scale devised for measuring their intensity was that developed by Braude.[8]

Negative—no erythema or induration.

Weak—smallest diameter less than 1.5 cm. with either erythema or induration.

Moderate—smallest diameter 1.5 cm. to 2.5 cm. with both erythema and induration.

Strong—smallest diameter greater than 2.5 cm. with both erythema and induration.

Violent—necrosis.

Preparation of tissue for histological study. A considerable amount of each specimen was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin for microscopic examination.

Results

Two hundred and fifty specimens were obtained for study (Table 1). These consisted of 93 fallopian tubes, 82 specimens of prostatic tissue, and 75 necropsy specimens consisting of liver, spleen, lymph nodes, and lung. All of these were cultured according to the method already described.

In six cases spheroidal colonies, translucent and slightly opalescent, were obtained. With age these colonies showed a definite brownish color. Stains revealed these organisms to be gram-negative cocco-bacilli of minute size. These bacteria produced no acid or gas with the usual sugars. All six of

these organisms agglutinated with polyvalent brucella antiserum.* Specimen 1 from prostate, agglutinated in titres up to and including 1:80; specimen 2 from prostate, agglutinated in titres up to and including 1:160; specimen 3 from prostate, agglutinated in titres up to and including 1:320 (the highest dilution used); specimen 4 from fallopian tubes, agglutinated in titres up to and including 1:320 (the highest dilution used); specimen 5 from a fallopian tube, agglutinated in titres up to and including 1:640 (the highest dilution used); specimen 6 from prostate, agglutinated in titres up to and including 1:640 (the highest dilution used).

TABLE 1

	Number of specimens	*Positive cultures*	*Per cent positive*
Prostatic specimens	82	4	4.9
Fallopian tubes	93	2	2.3
Autopsy specimens	75	0	0
Total	250	6	2.4

Using the methods described above for differentiating the species of brucella, we established that specimens 1, 3, 5, and 6 were Br. mellitensis, while specimens 2 and 4 were Br. abortus (Table 2).

The clinical and laboratory findings in these six patients are reported below. The clinical history, which was compatible with brucellosis in each case, was not obtained until after the cultures were known to be positive.

TABLE 2

	Prostate	*Fallopian tube*
Melitensis	2	2
Abortus	2	0
Suis	0	0

Patient 1. This 65-year-old colored male was born and reared in Shelby County, Tennessee. He had always lived on a farm, owned cattle, and consumed raw milk. In 1938, several of his cows aborted and a diagnosis of Bang's disease was made by a veterinarian. For the next five or six years the patient suffered from recurrent attacks of what he termed "typhoid-malaria," but no blood smears were examined for malarial

* The polyvalent brucella antiserum employed was that of Parke Davis and Company having a titre of 1:5240. A similar preparation was provided by Lederle Laboratories, Division of the American Cyanamid Company.

parasites. These attacks usually occurred in the spring or summer and were characterized by weakness, muscular aches, generalized low-grade joint pains, lassitude, and sweats. There were occasional dull, ill-defined headaches during this period. There was no history of a skin rash. The patient believed that he had a low-grade fever with very occasional chills. The only gastrointestinal disturbance noted during these episodes was anorexia.

On January 26, 1948, this patient entered John Gaston Hospital through the Genito-Urinary Clinic with the complaints of hematuria, difficulty in urination, polyuria, polydipsia, and some weight loss. Examination and study revealed diabetes mellitus, benign prostatic hypertrophy, pyelonephritis, and multiple abscesses of the left kidney. A transurethral prostatectomy and a left nephrectomy were performed.

Pathologic study of prostatic specimen. This specimen consisted of a number of small pieces of prostate. Microscopic preparation showed both a proliferation of the glandular tissue as well as the stroma. There was epithelial hyperplasia in many of the ducts and some of them were filled with small round cells. Through the stroma of the prostate were a number of eosinophils, lymphocytes, and macrophages. These were especially prominent around the blood vessels. An occasional eosinophil was seen in the wall of some of the smaller blood vessels. The endothelial cells of the bood vessels were swollen.

Examination six months after operation was entirely negative as to significant physical and clinical findings. A complete blood count, urinalysis, sedimentation rate, thick and thin smear for malaria, blood non-protein nitrogen, and fasting blood sugar were within normal limits. In view of the history of low back pain, even though it was felt that this was due to the genitourinary disease, roentgenograms of the vertebral column were obtained because of the reported frequency of brucella spondylitis. These were interpreted as negative as was a chest roentgenogram. An agglutination test for brucella was negative on August 8, 1948. the three intradermal brucella antigens gave strongly positive reactions.

Patient 2. This 76-year-old white male was born in Huntsville, Alabama, in September 1871 and lived there until 1890. He then moved to Bonham, Texas where he resided until 1910. At that time he came to Memphis and remained in this city. While in Huntsville, he owned cattle, but there was no history of Bang's disease. In Bonham, he also drank raw milk. Since his residence in Memphis, he has bought pasteurized milk. This patient stated that he had suffered from recurrent bouts of malaria since his residence in Bonham, though smears for malarial parasites were never made. These "malarial" episodes never produced clinical icterus. They were characterized by weakness, anorexia, muscular pains, malaise, intermittent headaches, and an occasional "dumb chill." These periods of illness tended to recur at intervals of six months to a year, with the last occurring in March of 1947.

This patient entered John Gaston Hospital on April 8, 1948, and was diagnosed as having benign prostatic hypertrophy, acute epididymitis, and arteriosclerotic heart disease. A transurethral resection of the prostate was performed and a rather prolonged convalescence ensued.

Pathological study of prostatic specimen. This specimen showed essentially the same histology as did the tissue from Patient 1.

Examination of this patient four months after surgery revealed no significant clinical

or physical findings aside from generalized arteriosclerosis. A complete blood count, thick and thin smear for malaria, and urinalysis were negative. Roentgenograms of the chest and spine were not significant. An agglutination test for brucellosis was negative. The three intradermal brucella antigens were strongly positive, with the Brucellergen giving a violent reaction.

Patient 3. This 74-year-old colored male was born and reared in northern Mississippi. He lived on a farm in that area, drank raw milk, and raised domestic animals until six years prior to his admission to John Gaston Hospital. At that time he moved to Memphis but continued to visit his former home quite frequently. For approximately the last thirty years this patient had frequent episodes of intermittent illness described as "malaria." No malarial parasites were ever demonstrated in the patient's blood.

On December 20, 1948 this patient was admitted to John Gaston Hospital with a history of acute urinary retention. A transurethral resection was performed, and the patient had a rather prolonged period of convalescence with persistent low-grade pyrexia.

Pathologic study of prostatic specimen. This specimen revealed essentially the same histology as that of the first two.

The patient was examined three months after being discharged from the hospital and aside from hypertensive cardiovascular disease no other significant findings were noted. A complete blood count, thick and thin smear for malaria, and a urinalysis were within normal limits. Roentgenograms of the chest and spine revealed minimal osteoarthritis. An agglutination test for brucellosis was negative. Two of the intradermal brucella antigens, the polysaccharide fraction and the protein nucleate fraction, were strongly positive.

Patient 4. This 74-year-old colored male was born in Memphis and lived there for eleven years. He then moved to Cahoma County, Mississippi, where he resided for thirty-five years. Following this, he returned to Memphis where he remained. During the period of his residence in Mississippi he lived on a farm, owned cattle, and drank raw milk. There was no definite history of Bang's disease among these cattle. During his later residence in Memphis he bought only pasteurized milk. The patient stated that when he was approximately 34 years of age, he had "typhoid-malaria" with chills, weakness, "muscle rheumatism," headaches, and anorexia. It should be noted that blood smears for malarial parasites were never done on this patient. Following this, he suffered from intermittent cramps in the legs and thighs. Also, after this episode the patient had periodic illnesses approximately every two years characterized by "dumb-chills," weakness, malaise, anorexia, and constipation.

On March 8, 1948 this man was admitted to John Gaston Hospital and a diagnosis of adenocarcinoma of the prostate with metastases and hypertensive cardiovascular disease was subsequently established. A prostatectomy and a bilateral orchidectomy were done.

Pathologic study of prostatic specimen. This specimen was composed of a number of pieces of prostatic tissue. Microscopic preparations of this tissue disclosed a diffuse prostatic carcinoma, an occasional cellular focus of lymphocytes, plasma cells, and an occasional eosinophil.

Examination of this patient five months after discharge from the hospital revealed pallor of the mucous membranes, hypertension, cardiac enlargement, as well as the

evidence of bilateral surgical orchidectomy and prostatectomy. There was roentgenographic evidence of metastatic involvement of the entire left half of the pelvis including the hip and upper femur. The chest was negative, the hemoglobin was 9.5 gm. per cent, and the urine showed a 2 plus albuminuria. Thick and thin smears for malaria and an agglutination test for brucellosis were negative. Intradermal skin tests for brucellosis were performed as previously described. All of these were negative except the Brucellergen which showed a moderate reaction.

Patient 5. This 52-year-old colored female lived in the delta of Mississippi until 1945 when she moved to Memphis. During her residence in Mississippi, she lived on a farm, owned cattle, and drank raw milk. There was no history of Bang's disease. This patient stated that in 1922 she had "typhoid-malaria" with chills and a high elevation of temperature each evening, headache, weakness, anorexia, sweats, and generalized muscle and joint pain. Blood studies for malarial parasites were never done. This illness lasted about one to two weeks; following this, intermittent recurrences of a similiar type were noted. These occurred especially in the spring and summer. There have been no such attacks since her residence in Memphis. Similar periods of sickness were said to have been suffered by her husband whose residence at present is unknown.

This patient was admitted to John Gaston Hospital on April 1, 1948 with the diagnosis of fibromyomata uteri, chronic pelvic inflammatory disease, and bilateral hydrosalpinx. A hystero-salpingo-oophorectomy was performed. This patient had a prolonged and stormy convalescence following surgery. At the time of discharge a low-grade fever was still present.

Pathologic study of fallopian tube. There was gross evidence of scarring in this specimen. The microscopic preparation showed extensive fibrosis with focal accumulations of lymphocytes and macrophages and some eosinophils. These collections of cells were scattered throughout but were particularly noted around the blood vessels. The endothelium of the blood vessels also showed some swelling.

Examination of this patient on August 26, 1948 revealed no significant clinical or physical findings. A complete blood count, thick and thin smear for malaria, and a urinalysis were within normal limits. Roentgenograms of the chest and spine were negative. The three brucella intradermal antigens yielded strongly positive reactions. An agglutination test for brucellosis was negative.

Patient 6. This 45-year-old colored female was born in northern Mississippi and for thirty-six years lived on a farm in this area. During this period she raised domestic animals and used raw milk. There was a questionable history of Bang's disease among this patient's cattle. For the past nine years she has lived in Memphis. However, she has continued to visit her old home and occasionally to drink raw milk. From about the age of 25 to 40 years, this patient suffered from recurrent bouts of "influenza." These episodes had no definite seasonal incidence and were characterized by frontal headaches, malaise, muscular aching, anorexia, occasional chills, and a persistent sensation of chilliness. Only rarely was a cough present.

On October 18, 1948 this patient was admitted to John Gaston Hospital with a history of lower abdominal pain of four months' duration. Pelvic examination revealed a fibromyomata of the uterus and an ovarian cyst. A total hysterectomy was performed.

The patient had a prolonged convalescence and showed a persistent low-grade fever each afternoon. This was still present at the time of discharge from the hospital on November 8, 1948.

Pathological study of fallopian tube. Grossly, numerous fibrous adhesions were noted. Microscopic examination disclosed essentially the same histological picture as that noted in the preceding specimen except that eosinophils were more numerous.

Examination of this patient two months after discharge from the hospital revealed no significant clinical or physical findings. A complete blood count, thick and thin smear for malaria, and a urinalysis were within normal limits. Roentgenograms of the spine and chest were negative, except for minimal evidence of osteoarthritis. An agglutination test for brucellosis was negative, but the three brucella intradermal antigens gave strongly positive reactions.

Discussion

Brucella organisms have been isolated in several cases of Hodgkin's disease and in numerous other pathological conditions. In some cases a possible etiological relationship has been suggested. It was believed that an explanation might be that in an endemic area, brucella may localize in chronically damaged tissue. Therefore, chronically injured tissues were cultured for these bacteria.

Two hundred and fifty surgical and necropsy specimens were examined bacteriologically for brucella organisms by a method based upon care in the collection and study of tissues. Identification of the organisms was made by (1) the morphology of the colony on tryptose agar, (2) the morphology and staining reactions of the individual organisms, (3) sugar reactions, and (4) agglutination with specific antiserum. Positive cultures were obtained from six surgical specimens. Four of these were from prostatic tissue and two from fallopian tubes. Two of the cultures from the four positive prostatic specimens were Br. melitensis and two were Br. abortus, while both of the positive cultures from fallopian tubes were Br. melitensis. Brucella were not obtained from any of the necropsy specimens.

Microscopic examination of multiple sections of five of the six positive specimens revealed a nonspecific chronic inflammatory reaction which was both focal and diffuse. The sixth specimen could not be adequately evaluated microscopically because of a widespread and overshadowing malignant process. A vasculitis was present which involved the small vessels—arterioles, venules, and capillaries. The endothelium of many of these vessels was swollen. Eosinophils were prominent in the positive specimens, being found in and about the small blood vessels as well as being scattered throughout the stroma. The eosinophils were more prevalent in the positive than in the negative specimens. A microscopic study of multiple sections of

the negative specimens revealed an occasionally similar, but less striking, tissue reaction.

The six patients yielding these positive specimens were investigated as to history, physical findings, and laboratory results. In all six cases there was a history of farm life and contact with cows and other domestic animals. In all cases there was a history of the consumption of raw milk. In one case there was a definite association with Bang's disease. In all cases there was a clinical record compatible with brucellosis. All six of these patients had a rather prolonged period of convalescence following surgery. All six cases gave negative agglutinations for brucellosis. Positive intradermal tests for brucellosis were obtained in all six cases. Four of the cases showed strongly positive intradermal reactions using three simultaneous brucella antigens. Only the polysaccharides and protein nucleate fractions were strongly positive in one case, and in another instance, only the latter was positive.

Summary

Two hundred and fifty surgical and necropsy specimens were examined bacteriologically for brucella. Brucella were obtained in six cases. Eighty-two prostates were cultured. Br. abortus was isolated in two cases and Br. melitensis in an additional two instances. Ninety-three fallopian tubes were cultured. From two of these, Br. melitensis was obtained. Seventy-five autopsy specimens were similarly studied with negative results. All of the positive surgical specimens showed gross and microscopic evidence of chronic inflammation. Eosinophilic leukocytes were prominent in the positive specimens. All of the patients from whom the positive specimens were obtained gave a clinical history compatible with brucellosis. In none of the six positive cases could specific agglutinins for brucella be demonstrated in the patient's serum. In all six cases intradermal brucella antigens gave positive reactions.

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THE PROBLEM OF THE LIPOID THROMBOPLASTINS*

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For a long time, it was accepted that calcium and cephalin activate prothrombin. There were secondary questions about the identity of the phosphatide and whether it was important that the phosphatide be bound to protein. Otherwise the issue was closed. Now the basic premise is in sufficient doubt to warrant a restatement of the problem in the light of newer observations.

" . . . I have come upon a fact which is of the very highest importance not only for the question of the coagulation of the blood but for the very much greater question of the nature of the chemical processes in protoplasm, which constitute life. This fact is that lecithin, a body omnipresent in protoplasm, can bring about coagulation." So wrote Wooldridge, in 1883.[23] The material he thus caused to coagulate was specially treated plasma from dogs injected with peptone. The plasma of peptonized dogs was also used by Howell[10] to assay the coagulant activity of his cephalin preparations. At that time, Howell employed one of Nolf's terms and wrote of "thromboplastic substances" as "the extractible substance or substances in the tissues which facilitates the process of clotting." This is considerably more general than his previous description[8] of "thromboplastic substance or thromboplastin, which neutralizes the antithrombin. . . ." Then, and ever since, "thromboplastin" has been a rather broad and variable term. It is now applied to diverse materials from fixed tissue or blood which accelerate coagulation, especially those which accelerate production of thrombin.

As discussed by Howell, a confusing set of reports had accumulated by 1912. One group of investigators dealt with heat-stable thromboplastins found in alcohol or ether extracts, while others used heat-labile, water-soluble preparations. Among the latter were Morawitz[20] and Mellanby,[19] who designated their aqueous extracts thrombokinase; and it was clear that they considered thrombokinase to be a direct activator of prothrombin.

If these old definitions are accepted, it may be considered that a thrombokinase would activate pure prothrombin, aided only, if at all, by calcium. In this sense, a thrombokinase is a specific kind of thromboplastin. There may be other thromboplastins which would not activate pure prothrombin.

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Howell preferred to consider that, basically, there was only one type of thromboplastin. He proposed that the heat-labile material was merely the thromboplastic lipid in combination with protein. However, Rumpf[10] believed that the two had distinct effects. Whereas the aqueous extracts were usually much more active than the lipoid extracts, the latter were reported to be more effective in the clotting of lipid-poor plasma. To this, an incomplete answer was offered by the assumption that the lipid was more effective when combined with protein. Today it is still frequently stated that prothrombin is activated by a lipoprotein.

In the years 1916-17, it was reported by Dale and Walpole,[2] by Douglas and Colebrook,[3] and by Heard[8] that trypsin accelerated blood coagulation. But the time was not ripe, and this remarkable finding was lost in the shadow of lipoid thromboplastin for two decades. The isolation of trypsin as a crystalline protein (not a lipoprotein!) by Kunitz and Northrop[15] offered a finer means for investigation. Promptly, Eagle and Harris[6] found that crystalline trypsin activated their crude prothrombin. Conversion to thrombin had also been accelerated by cephalin, with a similar crude prothrombin.[4]

Thus, Eagle[5] was confronted with a dilemma much like that faced by Howell twenty-five years previously. There were now two sharply distinct classes of thromboplastins: one represented by a crude ether-soluble cephalin derived from brain; one represented by crystalline trypsin. Which, if either, was a true thrombokinase? Eagle favored trypsin and was puzzled by cephalin.

Whereas trypsin is known to activate proenzymes, no lipid is known to do so. Therefore, it is more attractive to consider that trypsin is a direct activator of prothrombin, and that the thrombokinases found in blood and tissue preparations may be somewhat similar enzymes. As logical as this may seem, it has not been widely accepted. The principal arguments against this idea will be analyzed later in this paper.

Recently, Ferguson[7] has discussed the results of his detailed studies and added some experiments performed with Folch's subfractions of crude brain cephalin. These leave open the possibility that true cephalin is a potent thromboplastin, as Howell believed. To date, no clarification of the problem has been achieved by the purification of brain lipids, other than the separation of anticoagulants.

In contrast, as prothrombin is purified, it reaches a state where it is readily activated by trypsin, but not by crude cephalin. A purification program which has been in progress many years has offered repeated opportunities to elaborate on this finding.

The thromboplastin studied

The lipoid thromboplastin studied was similar to that used by Eagle[5] as crude cephalin. It represented that fraction of brain which was soluble in ether but not in acetone. As such, it contained, among other things, both cephalin and lecithin. Possibly there are diverse lipids and lipoproteins with distinct thromboplastic effects; and these thromboplastins may be accompanied by inhibitors. It is proposed to neglect these complexities temporarily and to focus on the main issue: Is this thromboplastin a direct activator of prothrombin? If not, then what else could be a direct activator? And how can one account for the undoubted acceleration caused by the lipid?

It should be mentioned, however, that this thromboplastin is different from the brain thromboplastin commonly used in clinical tests. Findings in this laboratory tend to corroborate those of Lewis and Ferguson[18] who reported that the first aqueous extract of acetone-dried brain contains protein factors of significance in coagulation. Leathes and Mellanby[16] had previously shown that an aqueous brain extract contained what they considered to be a kinase, precipitable at pH 5.5 and not soluble in ether. They further indicated[17] that the kinasic action was probably fortified by the presence of brain lipid.

In short, there is strong indication that an aqueous suspension of brain contains a thromboplastic factor in addition to the ether-soluble lipoid thromboplastin as used in this work.

It has occasionally been reported that lipid also augments thrombin activity. This raises the question whether the acceleration of thrombin production is spurious, due merely to a shift in the thrombin assays caused by the lipid. Results in this laboratory have agreed with the general experience that the thrombin is actually produced more rapidly, and that the effect is greater than could be accounted for on the basis of the observed shift in thrombin assays.

Preparation of the thromboplastin. The following is an outline of the most recent preparation: 150 gm. fresh calf brain was washed, minced, and triturated in a mortar for 10 minutes with 150 ml. acetone (analytical reagent). The acetone was decanted and trituration repeated successively with 5 more 150 ml. portions of acetone. The residue was suspended in 750 ml. ether (U.S.P.) and allowed to stand overnight. The ether extract was filtered and evaporated under vacuum at 35°C. The residue was twice extracted with 450 ml. portions of acetone for 15 minutes and finally dissolved in 100 ml. ether. This solution was evaporated under vacuum at room temperature and kept under suction for several hours to approach complete removal of the ether. The yellow residue, weighing 3.75 gm., was triturated with 37.5 ml. veronal-buffered saline, and the mixture was centrifuged, yielding a milky suspension which was stored frozen.

Lipid and prothrombin

It was observed repeatedly that the apparent activation of crude prothrombin by lipoid thromboplastin plus calcium depended upon the presence of contaminating material in the prothrombin. If the contaminating material was destroyed or removed, the prothrombin would lose its responsiveness to the lipid. Responsiveness to lipid could be restored by adding an appropriate protein fraction from plasma.

At the time these investigations were begun, the best available method for purifying prothrombin was that of Mellanby.[20] In this, the prothrombin was precipitated with the euglobulins, then extracted from the precipitate

with very dilute calcium bicarbonate. With modifications,[22] batches of 39 liters could be prepared at one time on a routine basis.

The calcium bicarbonate extracts were studied extensively. Fresh extracts were promptly converted to thrombin following addition of lipoid thromboplastin and calcium chloride. However, aged extracts were not activated by lipid plus calcium, although they were still activated by an aqueous extract of lung, as noted in 1938 (unpublished). Later the same year, Seegers *et al.*[31] reported that their prothrombin was activated promptly by lung extract but very slowly by cephalin.

The significant material contaminating the prothrombin could be destroyed or removed not only by aging, but also by heating or by adsorption of the prothrombin on magnesium hydroxide followed by thorough washing. Figure 1A, a 1939 experiment, shows that although the lipid did not activate heated prothrombin, it accelerated activation in the presence of prothrombin-poor plasma globulin. Figure 1B portrays a 1947 experiment. Here, the reagents had been prepared in a manner quite different from that of earlier years, as described elsewhere.[24] For reasons discussed at that time, the prothrombin-poor plasma globulin has been called prothrombokinase. In Figure 1B the pattern of results is the same; however, the apparent activation by lipid and calcium was not eliminated as completely as before.

It is apparent that the thromboplastic activity of the lipid depended upon at least one impurity in the prothrombin, which could be removed and restored at will. Moreover, the prothrombin plus its impurities produced thrombin without the added lipid, showing that the direct activator of prothrombin was present in, or obtainable from, the blood derivatives. This suggested that any effect the lipid had on production of thrombin was either indirect or secondary. For example, it might be considered that the lipid activated prothrombokinase and thus indirectly hastened activation of prothrombin.

Lipid and prothrombokinase

Collingwood and MacMahon[1] wrote of prothrombokinase in 1912, and proposed that it was converted to thrombokinase in the first step of a three-stage clotting mechanism. 1. Prothrombokinase → Thrombokinase; 2. Prothrombin → Thrombin; 3. Fibrinogen → Fibrin. Recently, the stages they postulated have been separated and used for a three-stage analysis.[24] In this, calcium is added to crude prothrombokinase and the mixture is assayed for thrombokinase activity at successive intervals. This technique makes it possible to chart the conversion process and, further, to enquire whether lipoid thromboplastin activates prothrombokinase.

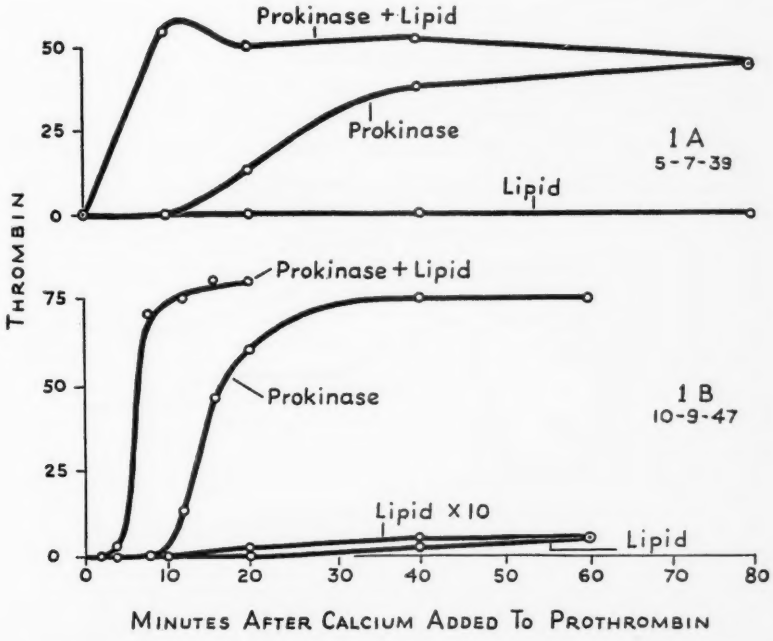

FIG. 1. Activation of prothrombin by prothrombokinase, lipoid thromboplastin, and calcium. All experiments were performed with calcium chloride present. Prokinase = prothrombokinase.

1A. For the experiment of May 7, 1939, the prothrombin was prepared by adding 0.9% of sodium chloride to a calcium bicarbonate extract, aging 3 days in the refrigerator, and heating in a 60°C. water bath for 5 minutes. The prothrombokinase was prepared from the residue left after extraction with calcium bicarbonate. The fresh residue was almost completely dissolved in a volume of oxalated saline equal to half the original volume of plasma, heated to 56°C. to remove fibrinogen, and treated with 21 gm. ammonium sulfate per 100 ml. The resulting precipitate was dissolved in distilled water equal to one-fourth the volume of original plasma and dialyzed against oxalated saline. Control tests showed no prothrombin in this reagent at the concentration employed. The concentration of lipid in the activation mixtures represented a 1/120 dilution of 1% stock suspension. The activation procedure was similar to that described in 1942.[22]

1B. For the experiment of October 9, 1947, prothrombin and prothrombokinase reagents were prepared and tested as described in 1948.[24] The most rapidly activated mixture contained 0.8 ml. prothrombin, 0.1 ml. prothrombokinase, 0.1 ml. of a 1/100 dilution of a 10% stock suspension of lipid and 0.1 ml. 0.0275 M calcium chloride. For the "prokinase" curve, the mixture was the same, except that 0.1 ml. buffer was substituted for lipid. For the "lipid" curve, buffer was substituted for prothrombokinase. For the "lipid x 10" curve, 0.1 ml. of a 1/10 dilution of stock lipid suspension was used, with no prothrombokinase added.

Results are shown in Figure 2. After calcium was added to prothrombokinase, there was a latent period, followed by a rapid increase in thrombokinase activity. When lipid was included there was a shorter latent period and a higher peak of thrombokinase activity. When ten times as much lipid was used, there was a pronounced elevation of the thrombokinase peak with relatively little effect on the latent period. Despite a rapid decline, the

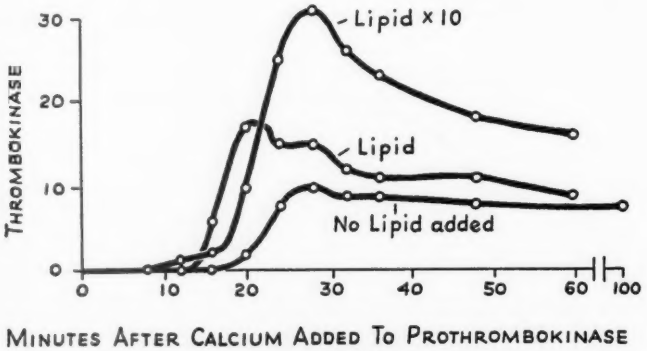

FIG. 2. Effect of lipoid thromboplastin on the prothrombokinase activation curve. The materials and methods were those previously described,[24] except that only one absorption with barium sulfate was done in the preparation of the prothrombokinase. For the "no lipid added" curve: 2.0 ml. prothrombokinase plus 0.2 ml. 0.0275 M calcium chloride. For the "lipid" curve: 1.8 ml. prothrombokinase, 0.2 ml. of a 1/100 dilution of a 10% suspension of lipid, plus 0.2 ml. calcium chloride. For the "lipid x 10" curve: 1.8 ml. prothrombokinase, 0.2 ml. of a 1/10 dilution of lipid, plus 0.2 ml. calcium chloride. As will be noted, the "no lipid added" mixture had 1.1 times as much prothrombokinase per ml. as the others. Separate experiments in which the prothrombokinase was constant gave the same pattern of results. The relative thrombokinase values were not reduced to percentage of an arbitrary standard.

thrombokinase activity remained, for the duration of the experiment, above that of the mixture with "no lipid added."

If the principal effect of the lipid were simply the direct activation of prothrombokinase, the curves should have risen from the origin and been about ten times as steep with the tenfold quantity of lipid. Since an autocatalytic mechanism or a chain reaction is involved,[28] an indirect effect, such as protection or enhancement of an autocatalytic factor, could account for as much shortening of the latent period as was observed. The materials were crude; and the explanation of the results would probably be complex. In

any case, the results do not encourage the view that the principal effect of the lipid is the direct activation of prothrombokinase.

Since the three-stage procedure is new and not fully developed, it is desirable to emphasize that it is nevertheless capable of detecting the kind of effect sought, i.e., a drastic shortening of the latent period with relatively little increase in peak activity. It was originally shown[24] that crude thrombokinase has such an effect on the activation of prothrombokinase. More recently,[27] a highly active protein fraction, which may represent a thrombokinase, has been described under the temporary name of "converter." At *high dilution,* the converter briskly accelerates activation of crude prothrombokinase without greatly elevating the activity peak. Such results make the converter a candidate for the role of prothrombokinase activator. Such results have not been obtained with lipoid thromboplastin.

Lipid and trypsin

It is now quite clear that thrombokinase can be obtained from slaughterhouse blood, although it may be necessary to convert it from an inactive state. The behavior of active thrombokinase is compatible with the possibility that it is an enzyme;[24] and it has usually been possible to substitute crystallized trypsin for blood thrombokinase.

The recent prothrombin preparations, as noted elsewhere, are suspected of containing a trace of thrombokinase or its precursor. Such prothrombins give a slight, limited response to lipid plus calcium. Beyond this low limit, the addition of more lipid does not lead to faster activation (Fig. 1B). In contrast, the prothrombin is activated with increasing rapidity when increasing amounts of trypsin are used (Fig. 3).

This last relationship must be borne in mind in the interpretation of experiments which purport to demonstrate that trypsin does not activate prothrombin. In Figure 3 it is seen that 0.1 microgram of crystallized trypsin did not activate the prothrombin reagent, neither did 0.1 ml. of twice-washed platelets. However, the two added to prothrombin together produced measurable thrombin. Experiments like this have sometimes been taken to imply that trypsin cannot activate prothrombin directly, but must act through the mediation of a blood factor, in this case platelets. That this conclusion is not necessarily valid is evident from Figure 3, where a tenfold quantity of trypsin caused activation without added platelets. In these experiments the range of trypsin concentration corresponded to a very slight degree of proteolytic activity as measured by the method of Kunitz.[14] As further illustrated in Figure 3, larger amounts of trypsin are rapidly destructive.

The necessity for testing several concentrations of enzyme was well recognized by Mellanby and Pratt;[21] therefore their negative results probably deterred subsequent investigators from pursuing the lead offered by trypsin. Using two-fold dilutions of pancreatic juice, they showed that dilutions just beyond the destructive one failed to activate prothrombin. No attempt was

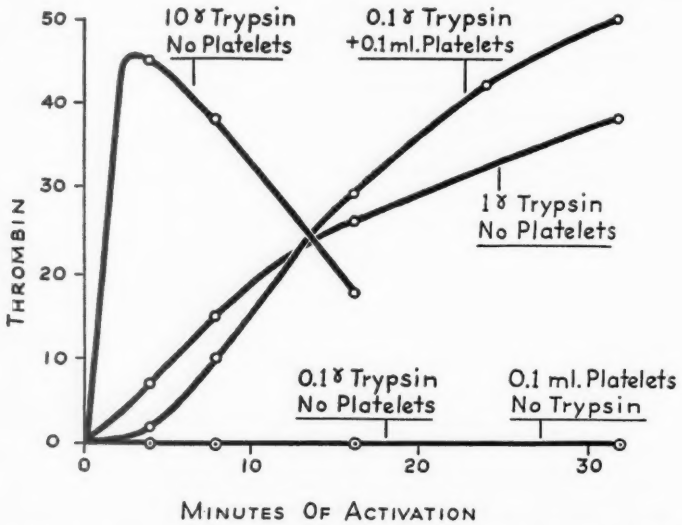

FIG. 3. Activation of prothrombin by trypsin, with and without platelets. 0.4 ml. buffer, 0.4 ml. prothrombin, 0.1 ml. platelets, 0.1 ml. Ca 0.0275 M., and 0.1 ml. trypsin (1/6,000, 1/600 or 1/60). Ca added 15 seconds before trypsin. Twice-washed rabbit platelets prepared as described.[25] One hundred and fifty mg. of crystallized trypsin, containing about 60 mg. protein (plus 90 mg. magnesium sulfate) were dissolved, dialyzed against 0.001 M HCl, and diluted to 10 ml. The figures on the chart represent the approximate amount of trypsin protein added to the activation mixtures.

made to determine how much of the destruction was due to chymotrypsin and how much to trypsin. So, it is not known whether an activating effect of trypsin should have been expected to dilute out further than the combined destructive effects of chymotrypsin and trypsin. It is known that their activated pancreatic juice contained considerable chymotrypsin, since the average specimen was said to clot milk quite rapidly. Trypsin does not clot milk, chymotrypsin does. It is conceivable that in their experiments there was sufficient trypsin to have an effect on prothrombin in conjunction with

other components of the clotting system. Their conclusion that trypsin releases an active form of thrombokinase may well be correct. But their results with crude pancreatic juice do not exclude the possibility that trypsin can also activate prothrombin directly. No one has yet claimed that crystallized trypsin cannot activate prothrombin.

If it is contemplated that prothrombin is ordinarily activated by an enzyme, it might be considered that the lipid could act as a cofactor. Such a relation of lecithin to the thrombokinase of daboia venom was considered by Leathes and Mellanby.[17] So far, attempts in this laboratory to show that the lipid can act as cofactor for trypsin have not succeeded. Experiments to be reported[28] have failed to show that the lipid acts as cofactor for crystallized trypsin in the activation of either crystallized trypsinogen or crystallized chymotrypsinogen. Little can be said about the lipid and the thrombokinase of blood.

Thus, the mode of action of lipoid thromboplastins remains an unsolved problem. However, the possibilities have not been exhausted. The heightening of the thrombokinase curve by the lipid offers one point of departure. It will be of interest to inquire whether lipid modifies production of thrombokinase, or certain side-effects, or the activity or persistence of the kinase. An entire group of possibilities is embraced by Howell's proposals[9-13] that the lipid would have no effect unless an anticoagulant were also present. Another such mechanism has recently been suggested by Jacox.[13]

Discussion

If the basic clotting mechanism is represented as three primary reactions:

1. Prothrombokinase $\longrightarrow$ Thrombokinase
2. Prothrombin $\longrightarrow$ Thrombin
3. Fibrinogen $\longrightarrow$ Fibrin

there are many things left unexplained. Among them is the mode of action of the lipoid thromboplastins. The foregoing studies on a given type of lipoid thromboplastin were designed to inquire how it impinged on the above basic mechanism. A concurrent review of the literature attempted to locate the origins of certain ideas and the evidence in their favor.

There is no good evidence that lipoid thromboplastin, in the presence of calcium, will activate either prothrombin or prothrombokinase. Nor is there proof that the lipid will act as simple cofactor for thrombokinase or for trypsin.

These views are derived from an extended study involving separation of the factors and reactions of blood coagulation. As these separations

progress, the problem may assume a different aspect. As one example, removal of impurities from prothrombin preparations has almost eliminated their susceptibility to activation by lipid, while responsiveness to trypsin was preserved. It cannot be said that, as further impurities are removed, the prothrombin preparations will not regain their susceptibility to lipid and/or lose their responsiveness to trypsin. The facts at hand do not suggest that this will happen. Nevertheless, it is obligatory to emphasize that the prothrombins studied here have not been pure. Furthermore, a thoroughly documented pure prothrombin has not been described anywhere. Additional uncertainties accumulate as consideration is given to the crudity of lipoid thromboplastins and to the question of lipoid anticoagulants.

As the phosphatide theory has become weaker, there has risen in its place the belief that lipoproteins may have thromboplastic effects which are qualitatively different from those of phosphatides. This is uncertain. The opinion that lipoproteins activate prothrombin is based on the fact that crude thromboplastic activity and lipoprotein are often found together and seem to be altered together by various procedures. However, as here shown the finding that a material accelerates activation of crude prothrombin does not constitute proof that it is the direct activator.

In the search for a true thrombokinase, the evidence in favor of lipoproteins is as weak as that for phosphatides. One must hesitate to accept a suspension of tissue particulates as a pure substance. Such material undoubtedly hastens production of thrombin in crude systems. But there has been no successful attempt to isolate its lipoprotein and to show that its lipoprotein activates prothrombin. Caution is further indicated with regard to the notion that the lipoprotein is an enzyme; for there seems to be no established example of a lipoprotein which is an enzyme.

Some of these common assumptions may eventually prove correct; but for the present, they should be recognized as opinions, with some suggestive support from experiments. Then, they will no longer obscure the more plausible view that thrombokinase, the activator of prothrombin, is an enzyme, possibly proteolytic, and quite possibly not a lipoprotein. For this, there is at least as much suggestive evidence, and the further advantage that the idea is in harmony with the general findings of biochemistry. Among the less attractive alternatives, the facts do not exclude a mechanism which requires the simultaneous presence of two or more biologic activators.

With a few exceptions, most recent investigators have turned aside from the lead offered by trypsin. This neglect has had its reasons. The work of Mellanby and Pratt seemed to be devastating and probably discouraged

many. But their experiments were uncontrolled for complications due to the chymotrypsin now known to have been present. Consequently their data do not exclude the possibility that trypsin can activate prothrombin. A further apparent setback occurred when the weight of evidence[32, 33] turned against the view that a fibrinolytic enzyme from blood can function as thrombokinase. This issue is not entirely settled. More important, there is no reason to believe that only one proteolytic enzyme is obtainable from blood. Finally, it has been difficult to demonstrate proteolytic activity where thrombokinase seemed to be present. But the present experiments show that only a minute amount of added enzyme, in terms of micrograms or of proteolytic activity, suffices to produce a large effect on the activation of prothrombin.

The evidence is not adequate to close the problem; rather it should be opened wider. Along with the hypotheses proposed in the past, more attention should be given to the possibility that prothrombin can be activated by one or more enzymes, and that this basic process can be modified in an indirect or secondary manner by phosphatides and by lipoproteins. The relative importance of lipoid thromboplastins in the physiologic control of the clotting mechanism is another question.

Summary

1. In the presence of calcium, either trypsin or crude cephalin seems to activate crude prothrombin. Which, if either, represents a direct activator of prothrombin?

2. Prothrombin is a proenzyme. Trypsin activates some proenzymes. No lipid is known to do so.

3. As prothrombin is purified, it reaches a state where it is readily activated by crystallized trypsin, but not by crude cephalin.

4. In the presence of platelets, one-tenth microgram of trypsin produced a large effect on conversion of prothrombin. Without added platelets, one microgram of trypsin readily activated the prothrombin. Thus, enhancement of trypsin effect by other factors does not prove that trypsin would be ineffective alone, provided the proper quantity was used.

5. The behavior of thrombokinase derived from blood is consistent with the possibility that it is an enzyme.

6. Lipoid thromboplastin accelerated production of thrombin from a mixture of prothrombin, prothrombokinase, and calcium.

7. In three-stage tests the lipid raised the peak of the thrombokinase curve, but caused only minor shortening of the latent period. This does not encourage the view that the lipid activates the prokinase. In contrast, three-

stage tests on a recently described protein fraction from blood have encouraged the view that it may activate the prokinase.

8. Experiments to be published have failed to show that the lipid acts as cofactor for crystallized trypsin in the activation of crystallized trypsinogen or crystallized chymotrypsinogen.

9. The problem has not been clarified by consideration of crude lipoproteins. There is no good evidence that lipoproteins activate prothrombin. There seems to be no established case of a lipoprotein which is an enzyme.

10. The actions of lipoid thromboplastins remain an unsolved problem. Several possibilities are yet to be explored.

Conclusion

Along with the hypotheses proposed in the past, more attention should be given to the possibility that prothrombin can be activated by one or more enzymes, and that this basic process can be modified in an indirect or secondary manner by lipids and by lipoproteins.

It is a pleasure for the writer to acknowledge his indebtedness to Dr. Milton C. Winternitz. The aid and complete freedom offered by Dr. Winternitz made it possible to resume this work at the end of World War II. His friendly interest and encouragement continuously fostered an enthusiastic attack on the problem.

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PERSONAL OBSERVATIONS

T. P. MURDOCK

It is well, and I think timely, that we state publicly our appreciation of men while they are living. It is difficult and perhaps impossible to describe Dr. Winternitz physically. His features are sharp, his head large, shoulders broad, and as to height, he is on the short side. Not infrequently men with this type of physical make-up are gifted with great drive. That he possesses this characteristic is not debatable. It is equally hard to describe him personally. At times he is cutting, quick, and sharp. Very sensitive people find him hard to understand. But when he "turns on the charm," he is delightful and companionable.

He came to Yale University Medical School as Professor of Pathology in 1917, having been trained by Dr. William H. Welch at Johns Hopkins. The department, as one would expect, soon became very active and assumed a very prominent place in the medical school activities.

Men can be trained for many positions in life, but administrators have it or do not have it. They cannot be trained. They are real leaders. He had it. Probably because of this characteristic, he was selected as Dean of the school in 1920 and continued in this position until 1935. The medical school reached a very high level during his regime. During this time he continued as head of the Department of Pathology. The dual task is always hard. He was able to accomplish it.

The new Sterling Hall of Medicine was occupied in 1923. This splendid plant was made possible largely through his stimulation and efforts. There were those who felt it was too large. It is accepted now that it is probably not large enough. It is so constructed that additions can be readily made. It will remain for many years as a monument to him.

He was and is a man of strong likes and dislikes. By the same token his friends are legion, his enemies of equal number. Those who know him well love him. I am amongst the latter group.

Many amusing incidences are told about him during the time he has been at the medical school. I recall several. Dr. David P. Smith and I went to see him, by appointment, relative to internes for the Meriden Hospital. There was a conflict in his appointments. Forgetting our appointment, he was meeting with another group. Their time extended well into ours. They were of the medical élite. We were beggars—beggars for internes. He had

provided tea and crumpets for the élite. Dave and I ate all the cakes. We were also beggars for food. To say that he was mad and to say that he cussed a little would be putting it mildly. We got our internes.

At another time the authorities of the New Haven Hospital felt it necessary to have the staff, above the grade of interne, licensed to practise medicine in Connecticut. Dr. Winternitz presented a bill in the legislature giving temporary licenses to these men. The hearing room was crowded with representatives of medicine from all parts of the state. Every man in the room registered his opposition to the bill excepting Dr. Winternitz and his attorney. The argument of the opposition was sound. It would have been special or class legislation. There he stood, alone, fighting for what he thought was right. I talked with him immediately after the hearing. He said, "I have been spanked before, and I will be again." He was.

I recall a talk which he gave to the State Medical Society at its banquet a few years ago. This had to do with a report of the postwar situation in Europe. He had been over there as a governmental representative. There was the usual cocktail hour before the banquet. Because of this some in the audience were inclined to be noisy—particularly one group in one of the corners of the banquet room. He did not look sadly at the group. He did not rap for order. He did not ask the toastmaster to rap for order. Instead he kept raising his voice, to drown out theirs, until it was almost a scream. He won out.

A peculiar situation has existed in Connecticut for many years, comparable to the feeling between "Town and Gown," in which the medical school has been looked upon by the medical profession with suspicion. Several reasons are given for this. Some thought the staff of the medical school looked down upon the general profession with scorn. There was probably a small element of truth in this. Others thought it was due to a full-time staff. (The wisdom of having a full-time staff was questioned by the profession for many years. It was felt that the teachers of clinical subjects were in competition with medical men in the field. It is now generally accepted that it is a necessity not only in undergraduate education, but also a requirement in general hospital staff organization for the proper training of internes and residents.) Still others have blamed Dr. Winternitz. I have been close to the situation for many years. It was clearly a misunderstanding, with both sides probably taking a narrow viewpoint. Fortunately the situation is being corrected and a very cordial relationship now exists.

Dr. Winternitz has been an untiring worker for Connecticut medicine. He, together with Drs. Herbert Thoms, Creighton Barker, D. Chester

Brown, and others, was one of the fathers of our Clinical Congress which is now accepted as one of the leading postgraduate courses in America. He has co-operated most generously with the program committees for the annual meeting of the Connecticut State Medical Society and the Clinical Congress by giving papers himself and by stimulating the younger men in his department to provide papers.

His work in pathology will live long after his productive years have terminated. In late years he has devoted a good deal of time and thought to vascular pathology. I recall some of his early writings in reference to the influenza epidemic of 1917 when he predicted an increase in pulmonary malignancy. This, of course, has come to be whether due to that specific cause or not. At present his influence is being felt in relation to the study of growth and of tumors.

His work as a teacher can best be shown by the men he has helped to train. I think of Dr. Harry Greene, Dr. Harry Zimmerman, Dr. Averill Liebow, Dr. Paul Rosahn, and Dr. Rolf Katzenstein.

He made a great contribution, as those of us who know him knew he would, to the war effort. He continued to run his department as well as to commute between Washington and New Haven weekly. At that time his office was under lock and key and he had the only key. His secretary, and those wishing to see him, had to wait in the hall until he arrived. There was no leak there. It is too bad that this could not be said about all governmental efforts of that time.

There are those who will say he is intolerant of the other fellow's viewpoint and that he plays favorites. Others will say that his viewpoint on medical education is a restricted one. I shall not. I shall say that he is an advanced medical educator, a splendid pathologist, a real administrator with great ability, both natural and acquired—and that I have great admiration and respect for him.

ACUTE TRAUMATIC SUBDURAL HYGROMA*

JAMES PETER MURPHY

A complication of head injury which is similar to, but distinct from the better known entity of subdural hematoma, is that of "subdural hygroma." This term was introduced by Dandy[3] to describe the appearance or development of abnormal collections of fluid in the subdural space. Dandy ascribed this condition to three possible etiologies: cranial trauma with leakage of cerebrospinal fluid, mixed with blood or plasma, into the subdural space through a "ball-valve" tear in the arachnoidal membrane; extra-dural infection, usually in the mastoid bone, with production of inflammatory transudate; and rupture of the ventricular system or arachnoid, again with leakage of fluid beneath the dura, in cases of chronic internal hydrocephalus. It is with subdural hygroma resultant from the first cause listed, head injury, that this article is concerned.

Wycis[9] published, in 1945, a report of seven personal cases of this condition and summarized all previous communications upon the subject of traumatic hygroma. Ninety-nine cases were listed in all. That there have undoubtedly been many more instances of subdural hygroma found operatively or at autopsy, recognized or unrecognized, is indicated by McConnell's[5] discovery of effusion beneath the dura in 32 of 63 cases of head trauma operated upon.

Payr,[7] in Germany, was first to describe what has come to bear the name of "subdural hygroma." His cases resulted from bullet wounds (World War I). Naffziger,[6] Cohen,[2] McConnell,[5] and Scott[8] made traumatic subdural effusions the subject of subsequent reports, indicating the surgical treatment and variations of the syndrome. Abbott, Due, and Nosik[1] reported the condition as a complication of blast concussion. The development of subdural hygroma in infants, again as the result of injury, has been brought to attention by Ingraham and Matson.[4] However, in spite of the numerous communications upon this subject, the condition is not generally thought of as a complication of head injury, whereas subdural hematoma immediately comes to mind when headache, drowsiness, slowness of the pulse, and mental and neurological phenomena develop after cranial trauma.

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In the ten histories which follow, the patients were operated upon with a preoperative diagnosis of subdural hematoma in all but two cases. Instead, hygromatous effusions in the subdural space were found in all, and in three cases the amount of fluid released was thought to be negligible. Prompt disappearance of symptoms of increased intracranial pressure and conversion of abnormal neurological findings in the last instances mentioned emphasize the morbid nature of even a few cubic centimeters of pathologic fluid in this otherwise virtually dry, "potential" subdural space.

Case reports

1. L. M., a 45-year-old white female, was admitted to the Emergency Hospital, Washington, D. C. on June 10, 1947. She was stuporous, with a scalp contusion in the left temporal region, and manifested a jargon aphasia and a positive Babinski response on the right. She had been struck by a streetcar shortly before admission. X-rays disclosed a linear skull fracture in the left temporal bone.

During the ensuing several hours, stupor deepened to coma, and the pulse slowed to 50. A subtemporal decompression was made on the left through an extensively macerated muscle and fractured bone. Incision of the dura allowed the escape of 100 cc. of straw-colored fluid. The underlying temporal lobe was contused.

Postoperative recovery was uneventful, but slow. Two and one-half years later the patient was employed as a clerk-typist and was asymptomatic except for a slight hesitancy in speech.

2. R. J., a 38-year-old colored male, was seen in consultation on June 11, 1948, because of frank papilledema of 4 diopters, with peripapillary hemorrhages. Four weeks before, he had sustained a head injury after having been thrown from a motorcycle and was hospitalized for ten days. Skull films revealed no fracture. Since discharge, he had developed excruciating headaches. Examination revealed a right abducens palsy and left hemiparesis, in addition to the papilledema described.

He was admitted to the George Washington University Hospital, Washington, D. C., and operated upon on June 14, 1948. Burr holes were placed in each parietal bone. Incision of the dura on the right resulted in the escape of about 4 cc. of slightly yellow fluid. One lateral ventricle was then tapped and air injected. Films made revealed a normal ventriculographic pattern.

The patient was operated upon with a preoperative diagnosis of subdural hematoma. The burr holes were closed and the patient was returned to his room. Postoperative relief of headache, diplopia, and staggering gait was so prompt as to be unbelievable. The patient was discharged on the 10th postoperative day, asymptomatic, and immediately resumed full-time employment. In retrospect, it became apparent that one was dealing with a minute, but morbid, subdural hygroma.

3. W. W., a 28-year-old white male, was admitted to the Washington Sanitarium, Takoma Park, Maryland, on February 27, 1949. He had been rendered unconscious in an automobile accident. There was a small laceration of the scalp. Skull films were negative, and there were no neurological findings.

Upon regaining consciousness, the patient complained of bifrontal headache, was drowsy, and became hallucinated. The vital signs were normal. Four days after

admission, a spinal puncture revealed an initial pressure of 170 mm., and there were many crenated red blood cells in a faintly yellow fluid. A positive Babinski sign had now developed on the right.

Burr holes were placed in each parietal bone, just above the insertion of the temporalis muscle. On the right, a small amount of xanthochromic fluid spurted forth when the dura was incised. The left side was negative. A small subtemporal decompression was effected on the right, the brain depressed with a flexible spatula, and a total of 15 cc. of liquid evacuated from the subdural space.

The next day, the spinal fluid pressure was 150. The patient was discharged from the hospital ten days after surgery, asymptomatic, except for mild headache and pain in the chest. He returned to his usual employment six weeks after release from the hospital.

4. S. D., a 45-year-old white male, was admitted to the Prince George's General Hospital, Cheverly, Maryland, on March 11, 1949, in a condition of semi-stupor, complaining of severe bilateral headache, and with a pulse of 44. Three days before he had been struck on the head and knocked out, but had regained consciousness. The symptoms described then developed. The extremely slow pulse rate precluded lengthy diagnostic studies, and without further ado he was taken to the operating room.

After trephining on the right, 15 cc. of yellow fluid were obtained from beneath the dura. The underlying brain bulged markedly. Therefore, a similar procedure was carried out on the left, and fluid of similar appearance spurted a distance of several feet into the air. With depression of the brain and irrigation, approximately 50 cc. were released from the space over the left hemisphere. The left burr hole was drained for 24 hours.

Recovery was slow but progressive. Six months later the patient was without symptoms and working full time.

5. E. B., a 44-year-old alcoholic white male, was admitted to the Alexandria Hospital, Alexandria, Virginia, on November 14, 1949, stuporous, confused, and with a positive Babinski in the right foot. Two days earlier, he had been injured in a brawl. After regaining consciousness, and during the ensuing 48 hours, he had complained of severe, generalized headache and had vomited.

Cranial trephines were made bilaterally, with release of 50 cc. of clear, yellow fluid from the left subdural space and 40 cc. of similar liquid from the right subdural space. The surface of the left temporal lobe was purple-red in color. Drains were left in for 24 hours.

The postoperative course was hectic because of the development of aspiration pneumonia and urinary tract infection. As these complications subsided and the patient became more alert, he presented a picture typical of Korsakoff's psychosis (confabulation being a prominent feature). At this time, transfer to a psychiatric hospital was considered. Large doses of vitamines were given, and several spinal punctures made. The mental status cleared, and the patient was discharged on the 26th postoperative day to return to work.

6. E. W., a 59-year-old white female, was severely injured in an automobile accident and admitted to the Emergency Hospital, Washington, D. C., on December 27, 1949. There were multiple contusions of the head and face and paralysis of the lateral rectus muscle of the left eye. The patient regained consciousness, but two days later became stuporous and developed a right hemiplegia with aphasia.

Exploring for a subdural hematoma, bilateral subdural hygromas (30 cc. each) were encountered and released. A ventricular cannula was inserted into the left parietal lobe, and at a depth of 4 cm. relatively fresh liquid intracerebral hematoma was found. Blood was withdrawn to a measurement of 30 cc. Drainage was instituted bilaterally for 24 hours.

Postoperatively, the patient regained consciousness but not the ability to move the right side or to talk. Within two weeks, evidences of a fistula between the left carotid artery and cavernous sinus became evident.

7. J. M., an 18-year-old white male, was seen in consultation on January 15, 1950, with complaints of left-sided headache of increasing severity since a fall down a flight of stairs 16 days before. There was slight sensory loss over the entire left side of the body. He was advised to enter a hospital for diagnostic study, but did not do so until he was brought to the George Washington Hospital, Washington, D. C., at midnight on January 21, 1950. At this time he was screaming with pain in the head and was almost completely paralyzed on the left side, with appropriate increase in sensory deficit.

The patient was so anxious and importunate for relief as to suggest a psychiatric condition. However, it was felt wise to rule out organic pathology. Accordingly, burr holes were made bilaterally; 2 cc. of straw-colored fluid were found in the right subdural space. The left side was negative.

As in Case No. 2, the paucity of findings (2 cc. of fluid) made one wish that further examination, including psychiatric evaluation, had been made preoperatively. Surprisingly, complaints and findings vanished postoperatively. Headaches subsided entirely, the hemiparesis cleared up, and it became apparent that the patient was alert and stable. He was discharged on the 10th postoperative day and returned to school.

8. F. P., a 51-year-old laborer, was admitted to the Emergency Hospital, Washington, D. C., on February 16, 1950, after having fallen four stories from a construction scaffolding, striking his head, chest, and forearm. X-rays revealed multiple fractures of the ribs on the right, traumatic hemothorax, fractures of the right wrist, but no fracture of the skull. There were numerous scalp lacerations, which were sutured. He had lost consciousness for about one hour.

The early part of the patient's hospital course was featured by the appearance of pulmonary complications and delirium tremens (he was found to be heavily alcoholic.) A traumatic hemothorax was aspirated on several occasions and vitamines, parenteral fluids, and whiskey were used in an attempt to control delirium.

The patient then developed severe headache, chiefly occipital in location, and became so drowsy and further hallucinated as to alarm members of his family and special nurses who were in constant attendance. On February 25, 1950, a spinal puncture revealed an initial pressure of 200 mm. of water, and mildly but definitely xanthochromic fluid. The plantar response on the left was questionable.

On the same day he was operated upon, under general anesthesia, burr holes being placed just above the insertion of the temporalis muscles. No more than 5 cc. of faintly yellow fluid were obtained by incision of the dura, depression of the brain with a spatula, and irrigation from the subdural space over the right hemisphere. The left side was negative.

The postoperative course was stormy and required further management of the chest injury and of symptoms of alcohol withdrawal. By March 13 he had become fully

oriented, alert, and quiet, and headache was completely absent. The left plantar reflex was now entirely normal, and there were no other neurological findings of interest. He was discharged seven weeks after operation, almost fully recovered.

9. R. B., a 16-year-old white male, was seen in consultation at the Leland Memorial Hospital, Riverdale, Maryland, on April 4, 1950. The patient had fallen from a moving truck the day before, striking his back and head. Momentarily dazed, he soon recovered consciousness, complained of pain in the mid-thoracic region, and was hospitalized. X-rays of the skull revealed a linear fracture parallel to the anterior one-half of the sagittal (interparietal) suture and extending into the region of the obliterated metopic suture. There was also a fracture of the 10th thoracic vertebra. At the time of examination the boy, a 220-pound athlete, was virtually uncontrollable, rolling around the bed in agony, screaming with bifrontal headache, alternately comatose and violent. There were bilateral Babinski signs, slight stiffness of the neck, and fullness of the retinal veins. The pulse varied between 52 and 70. The patient complained of "black vision."

A tentative diagnosis of subdural hygroma or hygromas was made, because of the absence of localizing signs and the obvious evidences of acutely increased intracranial pressure coming on so soon after a head injury. The skull fracture described seemed ideally situated to cause a "ball-valve" arachnoidal tear.

The patient was transferred to Prince George's General Hospital, Cheverly, Maryland. Spinal puncture was evidently impossible without the assistance of general anesthesia. As the state of consciousness deepened rapidly and the pulse slowed still further, cranial trephines were thought to be in order.

Incision of the dura on the right (temporal lobe) disclosed a very small amount (2 cc.) of yellow fluid and about 10 cc. of clotted blood. The brain herniated alarmingly. Therefore, a similar burr hole was made over the left hemisphere. Upon incision of the dura, faintly yellow fluid under tremendous pressure shot into the air for a distance of several feet. About 30 cc. of fluid were removed by brain depression and irrigation. A drain was placed into the subdural space on the left. A fairly large subtemporal decompression was then effected on the right; severely contused cerebral tissue (temporo-parietal) bulged into the opening.

Convalescence was slow but steady. The patient presented many of the mental symptoms resembling catatonia so often seen with cerebral contusion in children and adolescents. By the 10th postoperative day he was virtually normal, according to his parents, and he was soon discharged. At the time of writing, there has not been sufficient follow-up to prognosticate the future.

10. R. B., a 22-year-old white male, was admitted to the Prince George's General Hospital, Cheverly, Maryland, April 23, 1950, complaining bitterly of bifrontal headache and in a state of confusion associated with two major convulsive seizures. Thirty-six hours before, he had rolled over twice in a small automobile and was unconscious for an indeterminate period of time. Drowsiness and headache had developed slowly, and when convulsions ensued, he was brought to the hospital. There was no history of epilepsy prior to the injury.

X-rays of the skull were negative for fracture. Examination revealed a drowsy, belligerent individual, preferring to lie on his side with his hand on his forehead. The patient was mildly aphasic. There was slight weakness of the lower right side of the

face and a positive Babinski on the right. The clinical impression was "subdural hygroma."

Cranial trephines the same day disclosed about 5 cc. of clear fluid in the subdural space on the right (bulging brain) and approximately 30 cc. of the same type of fluid beneath the dura on the left. The left burr hole was drained for 24 hours.

Recovery was uneventful, and the patient was discharged as cured after examination in the office eight days following operation.

Discussion

The pathogenesis of subdural hematoma is well known since publication of the work of Putnam and Cushing, Kunkel and Dandy, and Munro. During head injury, especially polar (blows to the forehead or occiput), "snapping" of the brain, due to its relatively greater momentum with reference to the skull, results in laceration of veins draining into the dural sinuses. Blood escapes into the subdural space, remains loculated, and decomposes, and increase in osmotic pressure enlarges the hematoma by attraction of fluid. Membranes form, and eventually chronic compression of the brain, usually unilaterally, comprises the circulation of the brainstem so as to cause death if the hematoma is not evacuated.

The contents of chronic subdural hematomas not large enough to kill may, over a period of years, become crystal-clear in appearance. The writer remembers a specimen brought to the Brady Memorial Pathology Laboratory in 1940 from the Connecticut State Hospital (Middletown, Connecticut). It consisted of the brain and dural membranes obtained at autopsy from a patient who had been hospitalized for twenty years, with a diagnosis of "cerebral arteriosclerosis." Leathery membranes forming flat sacs filled with fluid of the consistency of distilled water overlay the cerebral hemispheres, both of which were compressed and indented. The case was obviously one of chronic subdural hematomas of many years' duration.

The phenomenon described in this article is not of this nature. It is of acute onset and brief duration, and complete lysis of blood elements cannot explain the clearness of the subdural fluid causative of symptoms and signs.

What is most probably true in the acute case is this: the same "brain-snapping" phenomenon referred to concerning subdural hematoma during head injury, instead of lacerating cortical veins, tears the arachnoid membrane in the vicinity of the arachnoidal villi, where it is most fragile, tenuous, and under greatest gravitational pull. Cerebrospinal fluid, with a little blood, escapes into the potential space between dura and arachnoid and continues to leak in, without absorption, in "ball-valve" fashion (Dandy). The total amount of such fluid, as noted, may vary from a few

TABLE 1

ACUTE SUBDURAL HYGROMAS RESULTING FROM HEAD INJURY

Name	*Age*	*Symptoms, signs*	*Hygromas*	*Size*	*Neurological complications*	*Treatment*	*Results*
1. L.M.	45	Coma, aphasia, + Babinski	Unilateral	100 cc.	Cerebral contusion	Subtemporal decompression	Excellent
2. R.J.	38	Headache, papilledema, abducens palsy hemiparesis	Unilateral	4 cc.	None	Burr holes	Excellent
3. W.W.	28	Headache, stupor, hallucinations, + Babinski	Unilateral	15 cc.	None	Subtemporal decompression	Excellent
4. S.D.	48	Headache, stupor, bradycardia	Bilateral	15 cc., R 50 cc., L	None	Burr holes, drainage	Excellent
5. E.B.	44	Headache, stupor, + Babinski	Bilateral	40 cc., R 50 cc., L	Cerebral contusions, vitamin deficiencies	Burr holes, drainage	Good
6. E.W.	59	Stupor, abducens palsy, hemiplegia, aphasia	Bilateral	30 cc., R 30 cc., L	Intracerebral hematoma, carotid-cavernous fistula	Burr holes, drainage	Poor
7. J.M.	18	Headache, hemiplegia	Unilateral	2 cc.	None	Burr holes	Excellent
8. F.P.	51	Headache, stupor, hallucinations	Unilateral	5 cc.	Cerebral contusions, delirium tremens	Burr holes	Good
9. R.B.	16	Headache, stupor, bradycardia	Bilateral	2 cc., R 30 cc., L	Cerebral contusions	Burr holes, drainage, subtemporal decompression	Good
10. R.B.	22	Headache, stupor, hemiparesis, convulsions	Bilateral	5 cc., R 30 cc., L	None	Burr holes, drainage	Excellent

to many (200 or more) cubic centimeters. In any event, an explanation of the production of symptoms and signs of increased intracranial pressure and brain-deficit when just a small amount of such pathological fluid is present in the subdural space must depend upon something more than compression of the brain by an enlarging mass of significant size. Apparently, the effusion in the subdural space then exerts a tamponade on the arachnoidal villi, preventing the resorption of cerebrospinal fluid produced by the choroid plexuses. This must be true; otherwise, such insignificant loculations as were present in Cases No. 2, 7, and 8 could not result in clinical neurological disease.

In the typical case of subdural hygroma, incision of the tense, bulging dura allows the immediate escape, under great pressure, of straw-colored fluid. The surface of the underlying brain is oftentimes cherry-red and otherwise discolored, manifestations of associated cerebral contusion. Depression of the available cortex with a flexible spatula allows the escape of further quantities of yellow fluid. The total amount of transudate-exudate in the subdural space, as noted, may vary from a few to hundreds of cubic centimeters. The protein content of this fluid may also vary widely (Wycis).

The only treatment is operative. Spinal puncture may be as dangerous in this condition as it is in epidural or subdural hematoma. Burr holes are best placed just below the insertion of the temporalis muscles, bilaterally, so that one or both trephine openings may be enlarged into subtemporal decompressions if need be. After dural incision has been carried out on both sides, thorough irrigation of the subdural space to remove retained and protein-containing fluid should be undertaken. Depending upon the capacity of the subdural space and the size of the hygroma, external drainage may be effected for 24 hours postoperatively.

The tendency of hygromas to be bilateral is at least as frequent as is the case in subdural hematoma, and this fact should not be forgotten at the time of operation.

Subdural hygromas in infants and children may be associated with compression of the cerebral hemispheres to a point where they fail to re-expand, or do so very slowly over a long period of time. Meanwhile, the subdural spaces (for the phenomenon is usually bilateral in the young) continue to pour forth unbelievably large amounts of clear fluid, obviously derived from a fistula in the arachnoid (cerebrospinal fluid). This condition persists until the arachnoid fistula or fistulae seal over by a process of

organization, or until the introduction of cicatrizing materials (Dandy) effects closure of the rent in the delicate membrane.

There is probably little of differential interest in a discussion of subdural hygroma, other than to point out that the amount of pathological fluid necessarily present in the subdural space to produce signs and symptoms may be very small, as in Cases No. 2, 7, and 8, and that diagnostic studies (ventriculogram) may therefore fail to reveal a large mass. It is obviously impossible to differentiate between hygroma and hematoma, preoperatively, given a patient who is developing pressure signs with or without indications of associated neurologic involvement, and the indication for exploratory trephines is urgent in either case.

One prognostic feature of interest is that in the case of subdural hygroma re-operation for relief of subdural tension is never necessary, whereas a certain percentage of patients from whom subdural hematomas have been evacuated by simple drainage have to be re-operated upon for removal of retained, solid, and organized clot.

There is no assurance, on the other hand, that long-term results in patients from whom hygromas have been drained necessarily will be superior to those in postoperative hematoma cases, even though brain compression of focal character is more likely with blood clot than with effusion. Four of the ten cases cited above suffered extensive cerebral contusions in addition to the formation of hygroma, and Case No. 5 was complicated by an intracerebral hematoma in addition to a carotid-cavernous sinus fistula. Personality change and the development of epilepsy are more dependent upon intrinsic brain injury than external pressure. Neither of these late results of head trauma has appeared as yet in the patients under consideration, but the passage of time since accident and operation has not been lengthy in any.

Summary

Ten cases of acute, traumatic subdural hygroma are presented. Severe head injury occurred in all. The ages of the patients varied from 16 to 59 years. In five, subdural effusions were found at operation bilaterally, and in five, unilaterally. Depression of the state of consciousness and severe headache were invariable clinical findings, and neurological abnormalities were quite common. The latter may as well have been due to associated cerebral contusion as to brain-compression. The quantities of pathologic fluid evacuated from the subdural space over one cerebral hemisphere in these cases varied from 2 cc. to 100 cc. Operative treatment consisted of cranial

trephine, with or without drainage for 24 hours, or subtemporal decompression. Re-operation was not necessary in any patient. The neurologic results were adjudged to be excellent in six cases; good in three; and poor in one (associated intracerebral hematoma and carotid-cavernous fistula).

Subdural hygroma simulates subdural hematoma in that it results from head injury, consists of the formation of pressure-producing fluid in the subdural space, and must be treated by operative evacuation and drainage. The pathogenesis of this condition is almost certainly that of tearing of the arachnoidal membrane along the superior sagittal sinus, and leakage of cerebrospinal fluid into the subdural space, sometimes with admixture of a small quantity of blood. Hygromas are indistinguishable, clinically, from hematomas. Complicating brain damage, principally cerebral contusion, is equally common in either condition. The immediate prognosis with subdural hygroma is better than that with subdural hematoma in that re-operation for evacuation of retained material in the subdural space is unnecessary.

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THE INFLUENCE OF ADRENAL AND PITUITARY HORMONES ON LIVER ARGINASE ACTIVITY FOLLOWING NEPHRECTOMY OR RENAL PEDICLE LIGATION IN THE RAT*

E. MYLON AND P. GOLDSTEIN

Impairment of intermediary nitrogen metabolism has been reported in dogs with reduced or abolished kidney function.[13] In nephrectomized animals fed a high protein diet, the deficiency is manifested by a 65-75% reduction of urea formation, by an early development of toxic symptoms, and by a short survival time.[12] No explanation is at hand for the influence of renal function on urea formation nor for the observed increase in toxicity.

On the basis of reports by Borsook and Dubnoff[2] and by Cohen and Hayano[3] concerned with transformation of citrulline into arginine in renal tissue, it was hypothesized that lack of the accessory renal source of arginine might decrease the plasma level of this amino acid and thus influence the urea cycle in the liver. This working hypothesis, as will be reported as part of this paper, has been invalidated by arginine determinations in plasma. Nevertheless, studies on liver arginase activity in rats, 72 hours after bilateral nephrectomy or 24 hours after bilateral ligation of renal pedicles, has demonstrated clearly a significant elevation of the activity of this enzyme.[11] Changes in liver arginase activity in normal animals have been previously related to pituitary and adrenal cortical influences.[5-8, 10] It was of interest, therefore, to investigate whether or not hormonal influences are involved in the great rise in liver-arginase activity that follows bilateral nephrectomy or renal ischemia produced by renal pedicle ligation. The paper to be presented includes the results of this investigation.

Material and methods

Male albino rats weighing from 250 to 400 grams were standardized on a Purina chow diet. The kidneys and adrenals were removed through a midline abdominal incision under light nembutal anesthesia (4 mg./100 gm. rat). Liver arginase activity was determined by a modification of the method of Hunter and Downs.[9, 11] At frequent intervals the accuracy of the method was tested by determining separately several 5 ml.

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aliquots of the same liver homogenate. In a typical experiment the following titration values and arginase units were found for normal fasting liver: (1) 4.9 mg. urea-N, 253 units, (2) 5.1 mg. urea-N, 262 units, (3) 4.6 mg. urea-N, 240 units, (4) 4.8 mg. urea-N, 245 units. The potency of the commercial urease was tested by its action on known quantities of urea.

Non-protein nitrogen was determined by the half-micro Kjeldahl technique on blood drawn by cardiac puncture at the time the animals were sacrificed.

The adrenalectomized fasted rats were maintained on ½ per cent saline alone. One ml. of Adrenal Cortical Extract (Upjohn) plus one mg. of 11-dehydrocorticosterone acetate* were given daily when substitution therapy was desired.

Partially purified crystalline growth hormone was prepared from beef anterior pituitaries according to the method of Fishman, Wilhelmi, and Russell.[4] The daily dose of this material was 5 mg. per rat, given subcutaneously in two divided doses. Plasma arginine concentration was determined with the method of Albanese and Frankston.[1]

Experimental results

A. Plasma arginine concentration in rats. A total of 18 rats was divided in three groups of six each. The first group were normal rats, three animals fasted for 24 hours and three for 72 hours; the second group were renal pedicle-ligated rats sacrificed 24 hours after ligation, and the third were nephrectomized rats sacrificed 72 hours after nephrectomy. Plasma arginine levels for the first group were found to be 1.672 ± .354† mg. per cent and 1.579 ± .179† mg. per cent respectively. The arginine concentration for the second group was 1.650 ± .397† mg. per cent and for the third 1.264 ± .231† mg. per cent.

B. Influence of bilateral adrenalectomy or cortical extracts. Reduction of liver arginase activity has been reported to follow removal of both adrenal glands.[5, 6, 7, 8, 10] Similar results have been obtained in a total of eight rats, divided in two groups of four each. In the first group (48 hours fasting) the average enzyme activity was 170.9 ± 6.3* units, in the second group (72 hours fasting) the activity was 148 ± 6.4* units, contrasting to an average of 256 ± 25.9* units previously observed in fasting normal animals.[11] Daily injections of cortical extract and 11-dehydrocorticosterone acetate for three days following removal of the adrenals kept the liver arginase activity in four rats at 253.6 ± 15* units, i.e., at normal values.

The influence of bilateral nephrectomy was studied in a group of five nephrectomized-adrenalectomized rats, fasted for 72 hours. The average enzyme activity was found to be 176.86 ± 26.7* units, contrasting to the average enzyme activity of 373.7 ± 46.2* units previously reported from

* Courtesy of Dr. C. N. H. Long.
† Standard deviation of the mean.

nephrectomized, non-adrenalectomized rats.[11] When four adrenalectomized-nephrectomized rats, fasted 72 hours, were treated with cortical extract and desoxycorticosterone acetate, as indicated above, the liver arginase activity

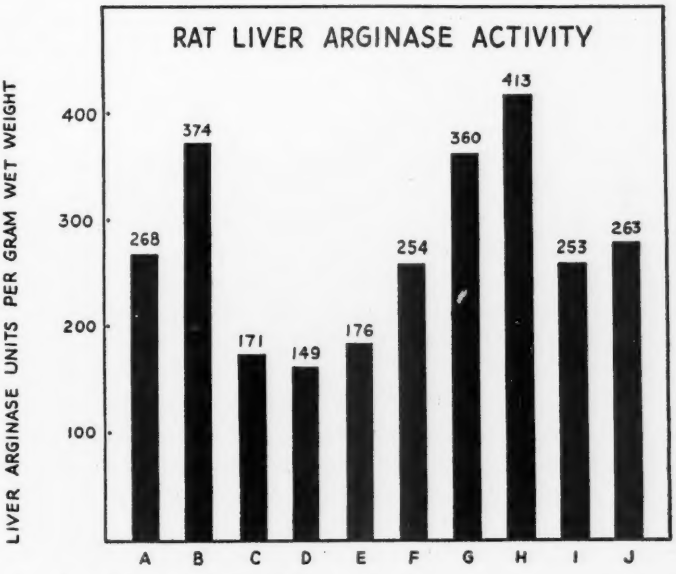

FIG. 1. Liver arginase activity in:

A. Normal fed rats.
B. Nephrectomized, fasted 72 hrs.
C. Adrenalectomized, fasted 48 hrs.
D. Adrenalectomized, fasted 72 hrs.
E. Adrenalectomized-nephrectomized, fasted 72 hrs.
F. Adrenalectomized treated,* fasted 72 hrs.
G. Adrenalectomized-nephrectomized treated,* fasted 72 hrs.
H. Renal pedicle ligation, fasted 24 hrs.
I. Renal pedicle ligation treated,† fasted 24 hrs.
J. Nephrectomized treated,† fasted 72 hrs.

* Treated with 1 cc. A.C.E. (Upjohn) plus 1 mg. dehydrocorticosterone daily.
† Treated with 5 mg. purified growth hormone daily.

rose to 359.5 ± 25.0* units, approaching the value of 373.7 units of nephrectomized non-adrenalectomized animals.

C. Influence of pituitary growth hormone. Subcutaneous administration of growth hormone to five renal pedicle-ligated rats, fasted for 24 hours,

resulted in an average liver arginase activity of 253.0 ± 24.7* units. This contrasts strongly with the average of 412.4 ± 51.6* units observed in untreated renal pedicle-ligated rats.[11]

Administration of growth hormone to eight nephrectomized rats (fasted for 72 hours) on the three days following removal of their kidneys likewise produced a marked decrease of the arginase activity. The average enzyme activity of 265.1 ± 24.6* units observed in these animals is a highly significant reduction from the average of 373.7 units obtained in untreated nephrectomized rats, fasted for 72 hours.

Concomitantly, the nitrogen catabolism in the animals treated with growth hormone was reduced as evidenced by the smaller rise in blood non-protein nitrogen. The average NPN of 126 mg. per cent after 24 hours, and 250 mg. per cent after 72 hours, in treated renal pedicle-ligated and treated nephrectomized rats, respectively, differs noticeably from an average of 158 mg. per cent and 350 mg. per cent, respectively, in untreated animals. Figure 1 summarizes the above results.

Discussion and summary

Arginine values determined in the plasma of nephrectomized and renal pedicle-ligated rats did not differ substantially from those obtained in normal rats. Under the conditions of these experiments, loss of the renal source of arginine did not reduce the plasma arginine level significantly. This result has invalidated the tentative explanation offered for the increase in liver arginase activity observed in nephrectomized and pedicle-ligated rats. Evidence presented in this paper relates the change in liver arginase activity at least in part to modifications of pituitary and adrenal action. It has been demonstrated that the increase of liver arginase activity in bilaterally nephrectomized or renal pedicle-ligated rats is lacking after the removal of the adrenal glands. Administration of pituitary growth hormone produces an effect like that of adrenalectomy.

These results suggest that nephrectomy or renal pedicle ligation may be accompanied by an increased output of cortical hormone or by a decreased activity of the pituitary growth hormone.

While the mechanism of this hormonal involvement is not yet known, it is further suggested that protein metabolites either of unusual composition or of abnormal amount are the agents responsible for the changes observed.

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* Standard deviation of the mean.

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SOCIAL AND GEOGRAPHICAL PATHOLOGY*

JOHN R. PAUL

For at least a century, and perhaps much longer, autopsy pathology has been a fitting introduction to the study of clinical medicine, and in like manner one might now say that *social pathology* becomes a fitting introduction to the study of social medicine and the whole list of disciplines it may include. Similarly *geographical pathology* may become a fitting introduction to the study of world medicine or world health and their disciplines, which would be a long list indeed. If, then, these new types of pathology have such important destinies, what are they, and what do they mean?

First, as to social pathology. Granted that the term implies the study of community disease, it should be early stated that it does not deserve the restricted interpretation sometimes attached to the word "social disease." Such usage would link it particularly to the study of delinquency, inebriety, vagrancy, and crime. This happily is not its meaning here.

As far as I can determine, the late Dr. John A. Ryle, Director of the Institute of Social Medicine at Oxford University, believed that social pathology represented that endeavor concerned with the diagnosis of disease existent within a given population or parts of that population.[5,6] He pointed out that this concept was far from new; that Sir John Symon (1816-1904) had the idea of social pathology clearly in his mind, and that the term was also employed by Alfred Grotjahn (1869-1931), the first professor of social hygiene in the University of Berlin, who made it the title of a textbook. Ryle set forth his views on social pathology in the language of the clinician he was—a clinician apparently about to perform a physical examination upon a community.† The methods of this physical examination were obviously concerned with measurements of the incidence and trends of diseases in a living community, of the frequency or absence of disease within component groups of such a community, and with correlations of this frequency with season, climate, race, and environment such as local housing, occupation, and other social conditions.

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† Ryle's concept of social pathology was the subject of a lecture given at the Institute on Social Medicine, in connection with the centennial celebration of the New York Academy of Medicine in 1947.[6]

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Dr. Ryle was accused by some of being a clinician who had rather naïvely discovered public health, but it is my belief that his accusers failed to recognize that his clinical wisdom gave him the right to extend to disease in the community his knowledge of disease in the individual, and to bring new types of judgment to bear on the situation. Others have tried this general plan. Sir James Mackenzie's Institute for Clinical Research founded at St. Andrews, Scotland, was perhaps another early expression of the idea conceived by a clinician. And, closer at home, the work of Dr. E. L. Opie and his co-workers on the spread of tuberculosis through families and among school children in Philadelphia was a contribution not only to the field of tuberculosis, but a fine pioneer example of the study of social pathology of tuberculosis in this country—this time conceived by a pathologist.

The efforts just cited were more than the mere compilation of data on disease incidence, of which we have had many whether they be derived from the records of reported cases of disease, from X-ray surveys, from serological surveys, skin tests, etc. The approach of the social pathologist calls for synthesis of these data. It is rather one of regarding the city as a patient and working up the facts as one might a case report.

Perhaps it is unnecessary to state how the community case report can be elaborated. In any community of appropriate size, the population can certainly be divided into various groups which, if occupational, offer the opportunity to study death rates or sickness rates among local farmers, doctors, stonemasons, or the unemployed, etc. Or the population can be divided into socio-economic groups in which the prevalence of duodenal or gastric ulcer, coronary disease, neoplasms, etc., can be determined in each class. This calls for a dissection of the social body and a clinical analysis of the findings.

These then are potential lines along which social pathology has been and may continue to be studied. It seems fair to predict that if such studies are fruitful, this new science can make a strong bid for recognition as one of the basic medical sciences. Actually, however, our definition of social pathology so far has been limited to communities and it requires little imagination to widen it to the concept of geographical pathology. This is merely taking the step from a smaller to a larger population, as from a county to a larger geographical area.

Geographical pathology is a much more venerable science. Its literature goes back to the treatise by Hippocrates, "On Airs, Waters and Places." By the eighteenth century it had become a subject for textbooks. Thereafter there appeared between 1850 and 1935 at least ten works on geographical

pathology[3] with that of August Hirsch in 1860 as perhaps the outstanding example. Hirsch had the perspicacity to document in the title of a two-volume work the fact that he was not only to be concerned with the geography of disease, but with its historical aspects in different places, thus indicating that the presence or absence of a given disease in a given place was wont to change.

But since the time of Hirsch, the cause for geographical pathology has not progressed very rapidly, although the International Society of Geographical Pathology was founded in 1929 and the subject did find support by medical historians such as Dr. Fielding H. Garrison and Dr. Henry E. Sigerist. But a stimulus came during the late war for obvious reasons. In Germany at the onset of hostilities in 1939, and probably before, steps were taken to prepare a *World Atlas of Epidemiology* under the editorship of Generals Zeiss and Rodenwaldt of the former German Army Medical Corps. This is known as the *Seuchen Atlas*. It appeared as a classified publication in 1942-1943, designed essentially to document areas of potential epidemic danger to invading troops, but it was also an important contribution to the geography of disease. In this country, military necessity also brought forth a medical geography book, namely, the first volume of *Global Epidemiology* by Simmons *et al.*[7] (limited so far to India and the Far East), which appeared in 1944 under the auspices of the Preventive Medicine Service, Office of The Surgeon General, U. S. Army. Contemporaneously, the subject began to and has continued to receive serious attention by the American Geographical Society.[3,4] It has become of concern to international organizations such as the World Health Organization, and according to Bulletin 11 of Science in UNESCO, Drs. Maurice B. Visscher and Gaylord W. Anderson of the University of Minnesota have now proposed the creation of an Institute of Geomedicine under the auspices of UNESCO and the World Health Organization. So it is obvious that there is a timely interest in geopathology in some quarters within a world which thinks hopefully in terms of united nations.

As for the scope of the subject of geopathology, like social pathology it is concerned with ways and means of detecting diseases in different places and peoples, of measuring their prevalence, and attempting to analyze the circumstances under which these diseases occur or are absent. It certainly should not be limited, as has been the case in the past, to the mere documentation of the presence of *infectious* disease nor should it be limited to the distribution of the etiologic agent causing any disease, but as Dieuaide has emphasized,[1] it should also include the *local* peculiarities of

all diseases. The manner, for instance, in which the pathological (and clinical) picture may differ in various parts of the world in pneumococcus pneumonia, rheumatic fever, and poliomyelitis infections, as well as cancer, are cases in point. In poliomyelitis, for example, a comparative twenty-five-year record might indicate that the same average annual case rate exists in three widely separated areas, representing the tropics, temperate climates, and the Arctic. But these equal, average rates per annum would not indicate that among natives in the tropics poliomyelitis is wont to be a sporadic disease of infants; in the temperate zones it is both a sporadic and epidemic disease mostly of school children; and in the Arctic there are more apt to be few epidemics, widely separated in time, in which all age groups may be involved. This is but one of many examples which might be chosen to illustrate the geographical peculiarities of disease.

Thus the functions of the geopathologist are not merely those of operating a clearing house for the records on disease prevalence which might be collected from various countries. Such methods were perhaps adequate in the days of August Hirsch but would not satisfy the demands of the serious student of geopathology today. Indeed, to do this sort of thing properly would almost require "Institutes of Geomedicine" as Visscher and Anderson indicate in their proposals relative to UNESCO and the WHO. It would require active investigations in far-flung areas similar perhaps to the local investigations demanded of the student of social pathology. The investigations of members of the International Health Division of the Rockefeller Foundation in their efforts to seek out the prevalence of jungle yellow fever in Africa and South America are examples. Thus new methods are and will constantly come to be available, so that one need not be limited for data or confined to the belief that the local official reports of diagnosed and reported cases of this or that disease in a given area represent the only or even an accurate means of determining the real local prevalence of a given disease and its trends, or the only means of studying geographical pathology.

But granted that the subjects of social and geographical pathology are interesting and provocative, why should we waste space here in attempting to expound these subjects? Why? Because we are more socially and globally conscious, and more "epidemiologically minded" than were our parents. And because recently there have been developed many new methods for determining disease prevalence which are now ready to use. If, for instance, poverty or affluence, hard work or indolence, warm weather or cold, moisture or aridity, crowding or isolation, or other micro- and

macroclimates breed disease, it would be good to know more about it, and it is not too much to hope that we can look to the pathologists of the future to tell of these new facts.

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DR. WINTERNITZ AND PEDIATRICS AT YALE*

GROVER F. POWERS

The Department of Pediatrics in Yale University was established on a full-time basis and as a major clinical discipline on July 1, 1921. Dr. Milton C. Winternitz was Dean of the School of Medicine and Dr. Edwards A. Park was founding chairman of the department.

The clinical service included patients with infectious diseases, as well as those with general pediatric disorders, and an out-patient clinic, shortly organized on an appointment basis for patients. There was a biological laboratory, under Dr. Park's immediate direction, devoted to the study of rickets and a clinical chemical laboratory under Dr. Alfred T. Shohl. Allergic disorders were studied and treated in a special clinic in which Dr. Ruth A. Guy (now Mrs. Francis R. Dieuaide) represented Pediatrics and Dr. James D. Trask, Internal Medicine. Dr. Ethel C. Dunham was chief of the pediatric out-patient service and Dr. Martha M. Eliot was resident on the in-patient service—both physicians later to distinguish themselves as officials of the United States Children's Bureau. The three internes were Dr. Ernest Caulfield (now Pediatrician-in-Chief to the Hartford Hospital), Dr. Joseph Weiner (practising pediatrician in New Haven), and Dr. Marian C. Putnam (now director of the James Jackson Putnam Children's Center in Boston).

On July 1, 1927, Dr. Park resigned to become Professor of Pediatrics in the Johns Hopkins University and the writer (who had come to New Haven with Dr. Park in 1921 but was at the time Pediatrician-in-Chief to the Henry Ford Hospital) succeeded him as Chairman of the Department at Yale. It is to emphasize the inspiration, vision, and wisdom of Dean Winternitz in the reorganization which occurred at this time that this tribute to him is offered with gratitude and appreciation.

Under Dean Winternitz and the new Chairman, the Department of Pediatrics continued its laboratory of clinical chemistry with Dr. John P. Peters of the Department of Internal Medicine serving as acting leader until the arrival of Dr. Daniel C. Darrow, a former house officer (1922-1923) but at the time (1928) a member of the staff of the Children's Hospital of St. Louis under Dr. McKim Marriott.

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The biological section which had contributed greatly to knowledge of bone pathology was discontinued, but a laboratory of clinical bacteriology was established under the direction of Dr. James D. Trask—a post held by him with great distinction until his untimely death (1942). In 1927 only a few pediatric clinics in the United States had facilities available for research and treatment of infectious diseases, the prime emphasis being at that time on nutritional diseases. Dr. Winternitz in his report to the President and Fellows for the year 1926-1927 said: "It became quite obvious that the nutritional field had been emphasized above that of other aspects of the subject [pediatrics], and that no pediatric clinic in this country was devoting attention commensurate with its importance to the field of infectious disease." The many contributions to science of Dr. Trask and his colleagues during the years 1927-1942 are testimony to the wisdom of the program envisioned by the Dean at the time of the reorganization.

At this time, also, another major development took place in the Department of Pediatrics—one of far-reaching importance. I refer to the activation of the desire of the late Dr. Arthur H. Morse, Professor of Obstetrics and Gynecology, that pediatricians assume responsibility for the babies in the Maternity Ward. Time has shown that many medical and psychological problems present themselves in the newborn period in respect to parents as well as to babies and that no similar area of pediatric experiences is of more vital importance than this in the training of the pediatrician. In his report to the President and Fellows for the year 1929-1930, Dean Winternitz recorded this development as follows: Pediatrics "is so associated with Obstetrics that the newborn child, together with the data accumulated by the obstetrician, is given over to the pediatrician for care. It is now planned to study the child's behavior at birth and to follow the individual so far as possible and advisable, and to give each child that enters the clinic the benefit of careful psychological as well as physical study." This is a dream further on the way to fulfillment through the activities of the Rooming-In Project and their integration, now in progress, with the activities of the Child Study Center. Dr. Winternitz thus had insight into what may prove to be the vital point for the development of sound mental hygiene and preventive psychiatry.

Psychiatry was ever in Dean Winternitz's plan for the enrichment of the curriculum of the School of Medicine; in his endeavors he had the wise and effective counsel of Dr. Arthur Ruggles. In his first report as Dean (1920-1921), Dr. Winternitz said, "It is necessary to look forward to the establishment of a section on Psychiatry"; and in 1922-1923 he remarked

in his annual report that "the teaching of Psychiatry at Yale has remained practically unchanged during the past twenty years or more, with practically no facilities, personnel, or opportunity to develop. Meanwhile, other schools have forged ahead. Indeed, psychopathic and psychiatric clinics are universally recognized today as essential units in a hospital-medical school organization, and the possible contributions to the welfare of the individual and community through the expansion of this field seem unlimited." By 1930-1931 a Department of Psychiatry under Dr. Eugene Kahn had been established and the Dean could write: "In Pediatrics . . . where a member of the staff of the department of Psychiatry and Mental Hygiene has been assigned . . . the opportunity has proved to be far greater than was anticipated, and it is believed that extension of this cooperation to other clinical services will be equally fruitful." The staff member was Dr. Marian C. Putnam, trained in pediatrics, psychiatry, and child development. At the time, although most medical schools had departments of psychiatry, there were few pediatric clinics oriented to the importance of studying and treating the emotionally disturbed child or observing the maturation of the personality of the normal child. Dr. Putnam was one of the first psychiatrists assigned to full-time duty on a pediatric service. Child guidance clinics were becoming more numerous and the Clinic of Child Development at Yale under Dr. Arnold Gesell had been making fundamental studies of mental growth and development for over a decade; the techniques of psychological evaluation based upon this work are widely used and appreciated both in day-to-day practice and in research. But many present-day concepts concerning the emotional reactions of children and the psychological aspects of their illnesses were held by only a few persons; Dr. Winternitz was one of these few. These concepts are reflected in the growing interest in child psychiatry, child guidance clinics, nursery schools, study homes for disturbed children, and in the assignment of psychiatrically trained personnel to pediatric clinics. Pediatricians more and more are realizing their responsibilities as family counsellors, confronted as they are by problems of child behavior which often stem directly from various types of parent-child relationships. Many pediatricians are well aware of this direction in which their professional life is expanding just as they are aware of its contraction in the domains of nutritional and infectious diseases.

The Rooming-In Project at Yale under the directorship of Dr. Edith B. Jackson (one of Dr. Putnam's colleagues and successors) supplies a framework for observations and studies of parent-child relationships in the prenatal period, the neo-natal period and early infancy—studies to be

integrated with those of a similar nature in later childhood in the Child Study Center under Dr. Milton J. E. Senn; both physicians hold joint appointments in Pediatrics and Psychiatry. These current developments stem directly from the early groundwork laid by Dr. Putnam and made possible by the active support of Dean Winternitz.

The Rooming-In Project further illustrates another concept often emphasized by Dr. Winternitz, namely, the integration of interdepartmental activities: Rooming-In demonstrates this integration in respect to pediatrics, obstetrics, nursing, hospital administration, and community services.

I have thus briefly indicated the major developments in the activities of the Department of Pediatrics at Yale in which Dr. Winternitz has played both creative and supportive roles. In less obvious but no less vital ways his interest, friendship, and encouragement have been of incalculable value and receive warm appreciation.

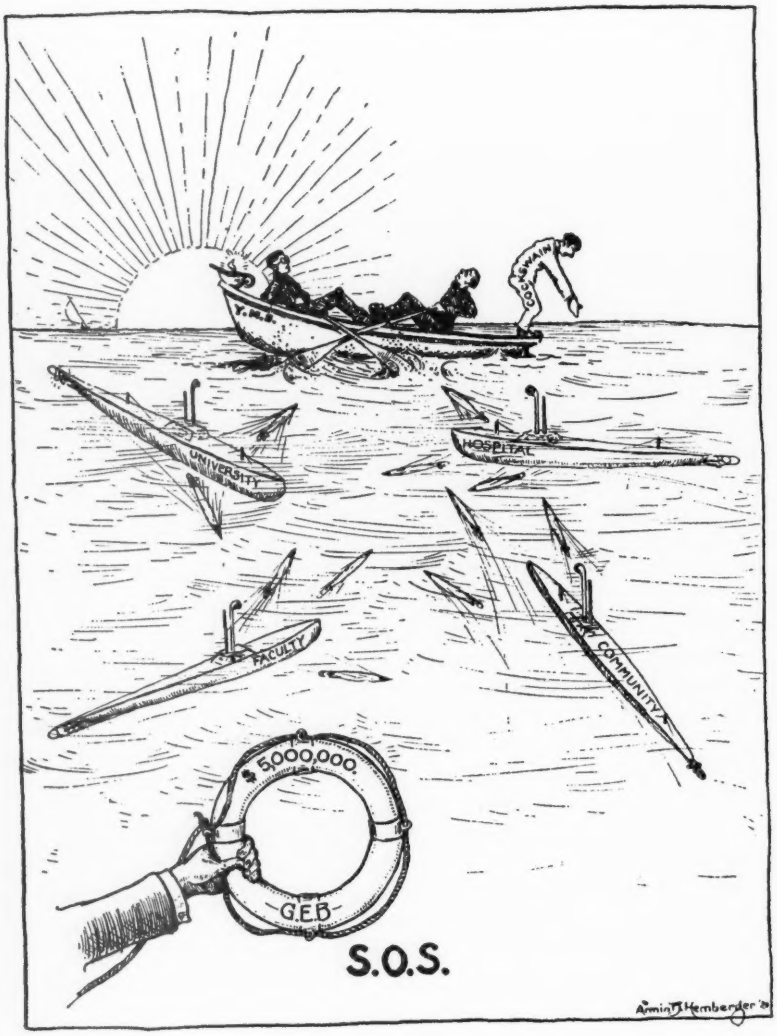
PSYCHIATRY AT YALE IN THE ACADEMIC YEAR OF 1925-26

ARTHUR H. RUGGLES

In the summer of 1925, a telephone call from the Dean of the Yale Medical School came to me asking for an opportunity to talk with me. I was on vacation at the time but the forceful Dean would take no postponement. We made an appointment to meet, in the near by city of Fall River, a few days later. At exactly the appointed hour, a quick-moving, forceful figure, in the summer costume of 1925, namely, linen plus-four's, appeared. He told me that he had purposely let the Department of Psychiatry at Yale die a natural death. Now he wanted to rebuild it as he had been doing with the whole Medical School for the previous few years. I think it is worthy of note that when Dean Winternitz went to the Yale Medical School, it was almost a sinking ship, and after some deliberation, instead of writing a long, detailed account of what he wanted to do in rebuilding, he sat down and with his own pen drew a cartoon of a sinking ship (the Yale Medical School), and sent it to one of America's great foundations. This unusual and dramatic appeal impressed them and a thorough study was soon made and the help of the Rockefeller Foundation was promised.

The Dean stated that he wanted me to come and help in rebuilding the Department. A less dynamic and enthusiastic salesman never could have interested me, as I already had two important professional assignments. The Dean's forceful, constructive, and enthusiastic picture of what he wanted to do and how he thought it might be done appealed to me at once, and before that interview was over I had promised to ask for a year's leave of absence to do what I could in the plan.

With the beginning of the academic term in September, 1925, I went to New Haven, began to give a few lectures to the students, and to look around for clinical material for teaching purposes. For several months that material had to be drawn from the wards and Out-Patient Department of the New Haven Hospital, and to me it was surprising that we could find almost every kind of psychosis and neurosis in the wards of a general hospital. The Dean proposed that we should take over one or two wards in the Hospital, have them reconstructed for psychiatric patients, and that they would be our clinical material. I felt that we would only be reproducing, in an inadequate building, what had already been done many times in provid-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ing hospital space and treatment facilities for a group of neurotic and psychotic patients. The Dean, with his brilliant, creative imagination, saw the point of this and said, "Then let's do something that will better fit the needs of psychiatry for the future." It was from that vision and with my unqualified approval that the plans for the Institute of Human Relations were developed. We estimated that such an institute, including psychology, child studies, research laboratories, as well as modern housing and treatment facilities for the neurotic and psychotic, would cost about seven million dollars. The Dean promptly made an appeal for this and he and I both thought that that amount had been virtually promised, but in a few weeks the answer came that the request had not been approved. I was greatly discouraged, but the Dean, with his usual forcefulness and his unqualified belief in the plan, suggested that we go over the plans again and see if they were in every way adequate. This study led us to the realization that we had made no provision for sociology in the multi-discipline institution, so we added space and increased the amount of money needed, and the dynamic Dean, with the new plans and the new version, went out and soon raised a sum considerably larger than the original seven million. I want to point out what a very great, farseeing plan this was and how the Dean would not compromise for anything second-rate. The Institute of Human Relations stands today and always will stand as a monument to the brilliance, energy, and courage of Dr. Milton C. Winternitz.

Small in stature, he is great in concept and achievement—always stressing research, scientific approach to the problems presented, and high standards of teaching, as well as the best possible care for patients—truly a modern giant in medical achievement.

Gradually the Department of Psychiatry developed into a real department, and slowly and despite many difficulties, the Institute was organized and built up. Many said that the Dean was a hard taskmaster. I say that he was a dynamic leader, unsparing in his own energies and those of others. It was only in this way that the development of a "sinking ship" into one of the great medical centers of the world was accomplished in approximately a quarter of a century. I worked with the Dean during that academic year and part time for many years after that, often from early in the morning until very late at night. It was only through his tremendous energy and self-sacrifice that the great work was achieved.

Dr. Winternitz has little tolerance for mediocrity. He at all times demanded hard work, a creative imagination, and thorough understanding

of the human problems. Demanding it of others, he never spared himself in these respects.

I shall always value my days and years of association with "Winter." It is one of the richest experiences of my professional life, and the Department of Psychiatry at Yale, and I, owe immeasurable stimulation and development to this little giant, who with his enormous drive has accomplished in his lifetime so much for scientific medicine, medical education, and the development of a dynamic psychiatry.

It may be fulsome for me to recount the tremendous achievements in chemical warfare that this pathologist brought to the Armed Services. He appeared before an Advisory Committee of the Secretary of War of which I was a member; and of all the great scientists who appeared before it, he was the only one who had the courage to state things as he saw them, had the remedies always at hand, and at times almost ruthlessly, but necessarily, brushed aside opposition, compromise, and mediocrity. I feel that the revamping of chemical warfare and the high state of efficiency to which our own Division of Chemical Warfare was brought, was, in the largest measure, due to the energy and brilliance of the former Dean of the Yale Medical School.

Long may he continue in his position of leadership in scientific medicine, especially in that part of it that stimulates young doctors to be the best of doctors and that brings to the sick and suffering the most modern of scientific understanding and treatment!

A DISCIPLE OF HUGH QUOIL TARRIES BRIEFLY IN NEW HAVEN

GEO. H. SMITH

For many years I have been intrigued by the personality of Hugh Quoil* and not a little amazed at the tale of his philosophy, or the seeming lack of a philosophy beyond the individualistic.

Hugh Quoil, soldier of Waterloo, came to New England in 1823 and by some chance found himself settled on the shores of Walden Pond some two miles from where Henry Thoreau had his abode during one phase of his study of nature. Quoil lived a life of peculiar pattern, defying colonial conventions and fostering his own notions as to what would make for his welfare. His ideas were essentially two: the first of these was never to pass beyond a supply of Concord rum; the second was always to take advantage of any opportunity to go fishing. These ideas afforded Mr. Thoreau a great deal of interest, but they imposed such severe restrictions upon Quoil's physical constitution that he succumbed before he could found a school of philosophy based on his fundamental, individualistic beliefs. Through such slight contact with Thoreau has Quoil's name found a place in history. Thus, it became a matter of mere chance that Quoil found a kindred soul who, under similar circumstances, might well have adopted his point of view—certainly with respect to his basic tenets. Perhaps some thoughts along these lines may serve to explain the more inscrutable behaviorisms which characterized the brief stay with us of Peter . . .

It is, indeed, true that Peter did not pass through the campaigns of Waterloo, but he did acquire, from many a fracas in Baltimore, scars of battle which could have been of credit to the original soldier. In 1917, Peter came to New Haven as autopsy-room attendant and janitor in the Department of Pathology. He was devoted to Dr. Winternitz, whom he held in highest esteem, although upon occasion he could not understand the workings of Winter's mind. But, like Hugh Quoil, he was resolved never to be separated for more than a minimum of time from alcoholic supplies.

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* All facts regarding Hugh Quoil are derived from *Men of Concord* by Henry D. Thoreau, Houghton Mifflin Company, Boston; The Riverside Press, Cambridge, 1936.

Also, he was determined to take full advantage of all fishing possibilities presented by the Connecticut shore.

Peter well knew that the five-gallon carboy of alcohol could best be opened with a hammer, thus providing an aperture which could not be sealed by any known or possible arrangement of cork and tube; consequently, there would be available a ready source of alcohol which, supplemented by that which could be salvaged through redistillation of all odd used portions found around the laboratory, could serve reasonably well as a never-failing source of inspiration.

Again and again, the first thing in the morning, Peter would be told that the autopsy room must be scrubbed, but Peter could not approach the task without first ascertaining the degree of satisfaction with which the still was operating. For this purpose only the taste-test could serve and, although the taste was adequate, in order to satisfy the conditions, it was essential, whenever possible, to have or at least attempt to have a period of somnolence in order that the finer points could be adjudged. Therefore it might be 9:30 A.M. when up and down the corridor would come the call for Peter.

"Peter, Peter, damn it! Peter!" Peter was not hard of hearing, he was just slow in hearing, and it would take some time for him to disentangle himself from among hundreds of oil paintings stored in the basement of Brady. (These paintings were of Chinese medical subjects and were a gift to the School in behalf of the noted Dr. Peter Parker in China.) The paintings formed an ideal place for temporary seclusion, and as Winter's shouts at last broke through his consciousness, Peter would slowly emerge from his resting place in response to the summons which seemed so imperative. Once on the straight-of-way he gained momentum and reached and passed Winter without pausing for more detailed instruction. The order which by now might be "Go to Prospect Street and take care of the muscovy ducks," went entirely unheeded. Peter unaided could interpret the word "go" which seemed wholly reasonable, but the muscovy ducks required further deliberation—toward which end Peter would seek well-tested advice and counsel. The result was that a half hour later Peter was not to be found either with the muscovy ducks or with the impedimenta for cleaning the autopsy room; in fact, he was not to be found in Brady Laboratory at all.

Broad Street at that time ran directly up the hill to Boardman, and on Broad Street were numerous swinging half-doors, behind one pair of which Rizzio was seated waiting to see what the day would bring forth. He would

agree with Peter that the idea of muscovy ducks was not to be entertained; in fact, one had only to look out of the door to see the heat shimmering above the pavement. Now, Rizzio was himself a confirmed disciple of Izaak Walton and, furthermore, he had much fishing equipment, essential for spending a day in that pursuit as prescribed by Hugh Quoil.

It required considerable searching to find Peter, with his equipment, gathering a mess of crabs on the shore at City Point or, with Rizzio, seated in a decadent coal pocket on the Kimberly Avenue bridge. From this divertisement he would be retrieved and led back to Brady to encounter once more the explosive wrath of Winter. The task of the day, however, had been so arduous that Peter's constitution required almost complete rest for the balance of that day. Hence he would retire to his room across Cedar Street and sit on the front steps which gave directly onto the sidewalk. At the foot of these steps there was a small triangle of earth wherein Peter assiduously cultivated the dozen or more petunias which represented his attempt at beautifying his environment and which furnished a bed into which he could tumble and develop his thoughts during the night.

Now Peter had been shocked at the price we were paying for guinea-pigs and he repeatedly affirmed the fact that he could raise guinea-pigs at less expense. He had acquired an animal cage on the understanding that he would care for the guinea-pig occupants, which he insisted must be white, pink-eyed, and with no tails. With these he was provided, but someone in the Department always arranged that the guinea-pigs be males, so that Peter's chances of demonstrating his laws of economics were without fruition. However, Peter's petunias thrived despite generally adverse circumstances.

The year 1917 and the following were years of great significance for the Yale Medical School. To "stop" or "go" were questions which occupied many a Committee and Board. In these discussions the "go" was always provided by Winter, and it was commonly believed that some of the dum-dum adjectives with which Winter favored the sedate and conservative members of the Committee were words he had not had a chance to use on Peter. It was necessary to keep Winter at or about the flash point, and for this Peter served better than any other agency.

It is not to be assumed that Peter did not render great service to the University during the period that he was with the Department of Pathology. There are few, indeed, who realize that Peter was one of the fundamental architects of the Yale Medical Library, for it was he who provided the shelves and boxes of books from which the shelves were filled—all this at

the instigation of Professor Winternitz. And how the Department could have survived the flu epidemic without Peter's assistance cannot be imagined. Consequently, it may well have been a misfortune that at about this time, a period of stress obliged Peter to gain his requisite stimulation from the museum which George Harley was preparing and which Winter regarded as so important in the records of Pathology. In any case, Peter was apprehended in an unusually flagrant diversion of alcohol designed for other purposes and he was summarily dismissed.

A CHALLENGING LEADER

EFFIE J. TAYLOR

Milton Charles Winternitz received his B.A. degree from the Johns Hopkins University at the age of eighteen years and thereupon entered the Johns Hopkins University School of Medicine. At this early age he began what was to be a brilliant career as a student of medicine, teacher, administrator, scientist, humanitarian, and philosopher.

Rarely is one individual endowed with outstanding proficiency in so many fields of human accomplishment, and rarely does one possess the acumen, the versatility, and the vision to sense the relationship between the creative and the technical in a world where no single factor exists as an end in itself. The realization of these interrelationships signifies an analytical mind and personality. To have the prophetic vision and the courage to pursue relentlessly the course of action foreseen, to inspire others who may not see eye to eye, and thus become a leader, is indicative of genius. Such marks the career of Dr. Winternitz.

I first recall Milton Winternitz as a fourth-year student of medicine. Our professional paths crossed when I was a third-year nursing student in the Johns Hopkins Hospital. In those early days some of the wards were in charge of student head nurses, and during my period as head nurse, Milton Winternitz was a medical student on the same ward and, with other members of his class, pursued a round of duties and responsibilities which brought the nursing and medical students into close relationship. We were a comparatively small group and knew one another well.

As a medical student, our Dr. Winternitz was an indefatigable worker, and looked upon his assignments with appreciation for the opportunities they afforded and, accordingly, his opportunities for carrying more important responsibilities were never lacking. This student inevitably would be found on the wards as soon as his classes were dismissed, and the hours he spent with his patients were not confined to those required to complete his duties as a clinical clerk. He was eager for opportunity to enlarge his experience and to obtain an insight into every procedure carried on in the ward by the internes and also by the nurses. He was ever inquisitive as he realized the physician had many outlets for his knowledge and many functions to fulfil. He firmly believed that medical students should know something about the nursing procedures essential to the care of patients.

Typhoid fever was then prevalent in Baltimore and cold tubs were the classical form of treatment. I recall that this young medical student would hasten to the ward to ascertain the temperatures of his patients that he might assist with these treatments if they were indicated. He was anxious to perform the procedure himself and he had many opportunities, since, during the season when typhoid fever was rife, the tubs required by the patients were legion. His capacity for keen observation and his frequent participation made him skilful in the use of his hands and he was disappointed if, by other assignments or class schedules, he missed participation in any treatment conducted either by the doctors or by the nurses. He became a great favorite with the patients as well as with the nurses.

In those days certain special wards in the Johns Hopkins Hospital were not segregated for service, but only for men and women. Within the same wards, medical patients were grouped in one section and surgical patients in another. Consequently, the students were often assigned for a considerable length of time to one group of wards. It was my privilege to observe the work of Milton Winternitz during part of his clinical experience in surgery as well as in medicine. Each medical student on the surgical service carried his equipment in a specially designed basket arranged in sections to contain the required supplies, and this basket the student had to keep in order. Medical students were responsible for making dressings on the patients under the supervision of the resident or the intern on the ward, and after they had received essential instruction and had attained skill, they assumed greater responsibility for the surgical care of the patients to whom they were assigned. The difference in the attitude of the several medical students toward the patients was noticeable. Some of them were impersonal and worked mechanically, with emphasis only on technique, but not so young Winternitz. He approached his patients as friends, and it was not long before he knew all about them and their families, with whom he frequently chatted during visiting hours. The patients looked forward to his visits and often asked when he was coming again. One of my colleagues, also a head nurse, told me that a patient remarked to her: "Just to see his head over the screen makes the day easier." Milton Winternitz was a methodical worker, and was never looked upon by the nurses as a burden. He did his share at all times and we admired him and were happy to have him assigned to our wards, not only because he was helpful, but because we recognized his brilliancy, his sincerity, and not less his sense of humor. He was full of pranks and one day dressed up one of the little five-year-old children to resemble an old man in a wheelchair, much to the amusement

of everybody on the ward. Nothing was too minor nor too insignificant to receive his attention when it contributed to the happiness and welfare of a person in need. One of his co-workers later remarked, "What a joy he would have been to patients had he chosen private practice; he had the gentle, sympathetic manner." But this student envisioned a broader field for his life work, as is evidenced by his career.

Dr. Winternitz was a student in the Johns Hopkins University School of Medicine during the regime of Doctors Osler, Halsted, Kelly, and Welch, and the influence and teaching of these men, together with his own unusual abilities, formed the foundation stones for his future career. The students who were privileged to come under these great masters were among the fortunates of their time. Not only did the students develop through the formal teaching which they received, but they had the unique privilege of knowing intimately these talented and distinguished physicians. The breadth of vision which characterized each of the masters, and the understanding, consideration, and respect they maintained for the patients were reflected in the teacher-student relations in the classroom and more especially in their frequent social and informal contacts. During the latter associations the differences between the master and the student disappeared, and all were encouraged to meet for discussion on terms of equality. Describing, in later years, the way that psychiatry was taught to the medical students in his student days, Dr. Winternitz with keen perception remarked, "Students learned from the example of the great clinical masters to approach the patient in a manner which imbued the latter with confidence and an optimistic outlook on life."

The influence of the four doctors descended not alone upon the students in medicine; the nurses were also recipients, and the attitude the masters assumed toward the patients was reflected without doubt in nursing as well as in medical care. It was always accepted in the Johns Hopkins Hospital that the nurses had the rights and privileges of education; they were not considered the nursing service of the hospital only. This interpretation was transferred at times to the medical students in definite terms. I recall while on one of the wards that Dr. Osler arrived unexpectedly for rounds with a group of sixteen to eighteen medical students. On seeing my hesitation to join the group, he asked that I accompany them as I might be needed, and turning to the medical students he instructed them to listen to what the nurses had to report, explaining that the nurses knew the patients better than the medical students did and that they had much to contribute. This was the normal spirit which prevailed; it was not without its effect on medi-

cal and nursing students alike. It was in an atmosphere of this kind that Dr. Winternitz was reared, and its influence fell upon fertile soil.

Such personal details are cited because of the Dr. Winternitz who was to be, and has now become. The characteristics he displayed, even as a student, foreshadowed the concept he embodied in the organization and administration of the Institute of Human Relations with which in later years he was so intimately associated. Those of us who knew him in his student days are not surprised that he was called to undertake one of the most difficult and constructive pieces of university medical school organization and administration in modern medical history, and that he excelled in his achievement because of his clear-sighted vision and his infinite perseverance and courage.

After graduation in 1907 Dr. Winternitz entered the Department of Pathology under Dr. William Welch, who early recognized him as a brilliant student with great promise for the future. In 1909, as one of his assignments, Dr. Winternitz began his instruction in general pathology and hygiene to the students in the Johns Hopkins Hospital School of Nursing, and he continued as instructor in these subjects until 1913. It was my privilege as a junior instructor in nursing to accompany the students to their lectures and laboratory exercises in certain of the medical sciences. This was my introduction to Dr. Winternitz as a teacher. I was impressed by the wealth of information he had to impart and how inspiringly and simply he developed a body of scientific material, presenting it in a way to be understood in its relation to the medical lectures the first-year nurses were then receiving.

Dr. Winternitz at no time relied implicitly upon notes, and the story is related by one of his listeners to a commencement address that he pushed his glasses above his forehead and become so engrossed and enthusiastic in what he was describing that when he desired to refer again to his notes, he did not know where his glasses were. In all probability he was not in the least disconcerted, and even if he did not again refer to his notes, I am convinced that he continued as if this were exactly what he had planned. He was an artist at thinking on his feet, particularly if he had room to walk to and fro as he proceeded with his discussion.

In the light of current practice, many may be amused at the idea of a policy which necessitated that a nursing instructor accompany nursing students to lectures or laboratory exercises given in the Medical School. The reason was not alone to provide a chaperone, but to insure that the nurse instructor in medical subjects would become conversant with the teaching

in science to enable her to interpret to the students certain facts not always understood by them. This practice was useful also in helping the nursing instructor to make the necessary correlation between the courses, since the time allowed was never long enough to permit of review, and the science content was usually crowded into too short a period of time.

Dr. Winternitz remained in Baltimore at the Johns Hopkins University Medical School as Associate Professor in Pathology until 1917. He was then invited to join the faculty of the Yale University School of Medicine as Professor of Pathology and Bacteriology. The School in New Haven was in the throes of a reorganization, and Dr. Winternitz had ample opportunity to bring into play his inherent ability and vision for the development of a medical school of the highest order.

He was appointed Dean of the Yale University School of Medicine in 1920, and from that time the growth and enrichment of the School were phenomenal. As a part of his concept for a new type of medical school with all the associated departments of the University open to the students of medicine, Dean Winternitz also envisioned a university school of nursing, and this school came into being in 1923. In his comprehensive report entitled "The Past, Present and Future of The Yale University School of Medicine and Affiliated Institutions," he said of the proposed School of Nursing, "This educational work should be under the control of the University through a director or dean. This director or dean should be the best available and obtainable educator in nursing." After searching the country for this superb educator, Miss Annie W. Goodrich was selected as the outstanding woman of her time.

Dean Winternitz also presented the need for a greatly expanded hospital with ample facilities for the better care of patients as well as for the education of students, and in due course his ideal was realized. While Superintendent of Nurses in the expanding hospital from 1923 to 1934, it was my privilege again to work with Dr. Winternitz and to participate in the reorganization of the nursing service in the New Haven Hospital. He was exceedingly helpful and appreciative of the problems during the difficult period when the Connecticut Training School for Nurses ceased to be the major unit in the nursing service of the hospital, and while the University School of Nursing was finding its rightful and newly conceived place in the same clinical departments.

Dean Winternitz felt that the relationships of the School of Medicine, the School of Nursing, and the Hospital were of primary importance, and accordingly he realized that policies which involved the three institutions

should be thoroughly understood and correlated, not only for the teaching of students in both schools, but in order to assure to the patients the most modern and perfect care. With these policies, the Dean of the School of Nursing and the Superintendent of Nurses in the Hospital, as well as the Hospital Superintendent, were in agreement.

The opinion that the School of Nursing should be established as a separate school in the University, with the same rights and privileges accorded to other professional schools, was held strongly by Dean Winternitz. He stated that it should be closely affiliated with the School of Medicine, but not an integral part of that School. He expressed the belief that every effort should be made to co-operate in so far as the available personnel in one faculty might be of assistance in the teaching of the other, but the Nursing School must assume responsibility for working out the content and the direction of its development for nursing education.

In addressing the National League of Nursing Education in New York City in 1935, Dean Winternitz made a sharp distinction between "nursing for education and education for nursing." He expressed the conviction, which he has indicated as essential for medical education, that well-prepared students would work and work hard for the knowledge needed to fit them for their professional careers. This declared opinion was in defense of his theory that didactic work should not be evaluated on the basis of fixed schedules of hours of required work alone. He advanced the thought that nurses should have more freedom than was traditionally conceded to develop their own programs of study, believing that if a student is doing what she wants to do, she will need no coercion.

Dean Winternitz has frequently said that in his judgment nursing students should not be depended upon, any more than are medical students, for expert work in the hospital and, moreover, that the argument for an internship and residency holds as good in the preparation for nursing as it does for medicine. He has expressed the opinion that the principles of teaching which apply to medical education in all probability hold as well for nursing, and he has indicated that the education of the nurse should be broad enough to prepare her not only for the practice of nursing, but also as a teacher and interpreter of nursing in its many aspects and relationships. With this, nurse educators are wholly in agreement. He has consistently advocated that teachers of nursing students in the various biological and social sciences be prepared as specialists in order that they may know what body of knowledge should be included in their teaching. These teachers may be nurses who are also skilled in the essential medical content or they may

be doctors who are well informed of the content of the sciences required for nursing. In either case, specific training and education are essential to successful instruction.

A vision for nursing education on the broadest lines compatible with the function of nurses as required for the bedside care of the sick, the prevention of illness, the community relationship and its environmental aspects, has long been held by nursing educators, but not always by educators in medicine. The functions of teaching, and research into the nature of nursing with its many sided facets, have been kept well to the fore in the thinking of those most concerned with the preparation of nurses for their greatest usefulness to humanity. The most inhibiting handicap to achieving the goal has ever been lack of financial support. This support must be found before the ideals advanced by nursing itself, and so ably on many occasions expressed by Dr. Winternitz, will become a reality.

On this occasion it is my joy and privilege to express appreciation to Dr. Winternitz for the debt we owe to his far-sighted vision in nursing education not less than in medical education. The influence of his support of this School of Nursing has spread throughout the world, and nursing offers him profound gratitude.

As one who has known Dr. Winternitz for almost half a century, I am happy that at the beginning of our professional lives, and at later intervals, we had the opportunity of working together, and it gives me joy to add, in this his year of retirement, my personal appreciation for his magnificent accomplishments. These will continue to shed their influence until in time the full significance of their meaning will be within the horizon for medicine and for nursing.

THE USE OF BLOOD VOLUME STUDIES AS A CLINICAL AID IN INTRAVENOUS THERAPY*

ROBERT TENNANT, LESTER ADELSON, GEORGE McADAMS, AND ROBERT DAVIE

In any hospital in which there is an active blood bank, clinical problems dealing with the adequate maintenance of the fluid as well as the cellular components of the circulation are continually encountered. Many instances have occurred where patients have received inadequate replacement therapy, but equally serious have been the times when excessive use of blood, plasma, or fluid has resulted in deleterious effects on the patient. It has been customary to rely on the hemoglobin, hematocrit, serum protein, or "clinical judgment" to arrive at the correct solution to these problems.

Through the work of a number of investigators, notably Gregersen[3] and associates, it has been established that the total blood volume is remarkably constant in health, but with the development of disease states marked alterations in blood volume may ensue. Furthermore, these changes in total blood volume may be completely masked through shifts of fluid and proteins between the vascular and extravascular spaces. Determinations of the hematocrit, hemoglobin, and serum protein, which are only measures of concentration, will under such circumstances give little notion of the total amount of these components in the circulation and at best will be poor indices of the patient's condition. The calculation of the total volumes of plasma, red blood cells, and plasma proteins and comparison of these values with standard values for normal healthy individuals permit a more rational approach to adequate and quantitative replacement therapy.

The extensive clinical and experimental investigations of Gregersen,[3, 4] Clark,[2] Beling,[1] and others employing the blue dye T-1824 (Evans Blue) have demonstrated that it affords a reliable and reproducible clinical method for the estimation of plasma, red blood cell, and total blood volumes. We therefore have used this method to aid in planning intravenous fluid therapy and to evaluate its usefulness as a practical method adaptable to use in the average general hospital.

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Methods

The methods used are similar to those described by Gregersen in 1944.[3] Fifteen cubic centimeters of blood are withdrawn without tourniquet, if possible, from the antecubital vein and placed in two tubes, one containing heparin and the other KNH_4 oxalate. The entire contents of an ampule of Evans Blue dye (5.3 cc. of a .5% sol.)* are then injected through the same needle which has been left in place. Fifteen minutes after the injection of the dye a second sample of blood is drawn and placed in another tube containing heparin. This is a slight departure from Gregersen's method.[3] The optical density of the dye-tinged plasma is measured with a Coleman Junior Spectrophotometer using a filter with wave length of 620 μ. The concentration of dye in the unknown is determined by comparison of its optical density with those of previously determined concentrations of dye in plasma. The total plasma volume is determined from these data. Hematocrits are determined by the Wintrobe tube method and the plasma protein concentration by the Phillips copper sulphate falling drop method.[8] From these data the total blood volume, total plasma volume, total red blood cell volume, and total circulating protein are estimated. All of these values are divided by the weight of the individual in kilos to give volumes per kilo of body weight. This simplifies comparison with normal values when calculating deficits or excesses.

Before making evaluations of patient's needs it was necessary to determine the values for blood volumes in normal healthy males and females. Accordingly, blood volume determinations were carried out on 30 males and 30 females. Individuals of a wide range in height and weight were used so that the mean values would be based on a good representative cross section. The mean values with standard deviations of the mean are presented in Table 1. These values have been used as the baseline for determination of the blood requirements in all of the subsequent work. Deficits or excesses were calculated by taking the difference between these mean values and the actual values determined in a given instance. The deficit or excess (expressed in cc. per kilo) was multiplied by the patient's weight in kilos to give the total deficit or excess.

In Table 2 the values for blood volumes as determined by a number of investigators are presented together with the present values. It is evident from these data that the various methods employed yielded somewhat different but equally reliable results. Differences in the normal values depend on variations in technique and instruments used and emphasize the importance of determining one's own normal values before applying the methods to the study of patients.

During the past two years these methods have been used in evaluating the blood volume status of some three hundred patients presenting a variety of

* Ampules of dye may be obtained from William R. Warner and Co., Inc., New York City.

clinical pictures. From these we have chosen a series of cases to illustrate typical situations in which blood volume studies have aided materially in clarifying the problem of intravenous therapy.

TABLE 1

NORMAL VALUES, HARTFORD HOSPITAL

	Males (30)	*Females (30)*
Plasma volume, cc./kg.	41.9 ±5	40.2 ±4
RBC volume, cc./kg.	34.5 ±4	27.9 ±3
Total blood volume, cc./kg.	76.4 ±8	68.1 ±6
Total plasma protein, gm./kg.	2.95 ±0.4	2.90 ± .3

TABLE 2

BLOOD VOLUME DETERMINATIONS ON 30 NORMAL MALES AND 30 NORMAL FEMALES AT HARTFORD HOSPITAL COMPARED WITH FINDINGS OF OTHER AUTHORS

Author	*Method*	*No. subjects*	*Plasma volume cc./kg.*	*RBC volume cc./kg.*	*Total blood volume cc./kg.*	*Plasma protein gm./kg.*
Hartford Hospital	Dye	30 M 30 F	41.9 ±5 40.2 ±4	34.5 ±4 27.9 ±3	76.4 ±8 68.1 ±6	2.95 ±.35 2.90 ±.30
Gregersen[3]	Dye		45.0 ±4.0		85 ±8.9	
Kelly[5]	Radioactive phosphorus, P^{32} 9		36.04 ±4.69	33.94 ±4.87	69.81 ±7.79	
Keith*	Dye	42 M & F	50.0		85.0	
Gibson*	Dye	49 M 41 F	43.08 41.5	34.62 24.6	77.7 66.1	
Chang*	CO	16 M & F			66.2	
Hopper*	CO Dye	9 M & F	45.3 45.5	34.9 35.0	80.2 ±5.5 80.5 ±8.6	
Smith*	CO Dye	20 M & F			71.0 ±5.0 93.0 ±8.0	
Hevesy*	P^{32}	21 M & F		38.8		
Nylin*	P^{32}	19 M & F		33.7		

* Figures are found in the article by Kelly *et al.*[5]

Case I. D. H., a while male, aged 49, entered the hospital complaining of periodic attacks of right upper quadrant pain and jaundice of two years' duration. A diagnosis of gallbladder disease with common duct stone was made and on November 17, 1948, cholecystectomy was performed. Stones were removed from the common duct. The patient contined to be jaundiced and he was re-explored on December 1, 1948. At

operation extensive bleeding was encountered and the patient went into profound shock. Fifteen hundred cc. of blood were given in the operating room. The patient was still in shock four hours later at which time the blood volume was determined (Chart 1). The findings showed a 40% deficit in the total blood volume although the hematocrit was 40 and the serum protein 7.0 gm.%. These gave no indication of the 2700 cc. deficit which was present. The patient received 2500 cc. of whole blood and 500 cc. of plasma in the next 48 hours, with recovery from shock. A repeat blood volume one week later showed essentially normal status with hematocrit and serum protein still unchanged.

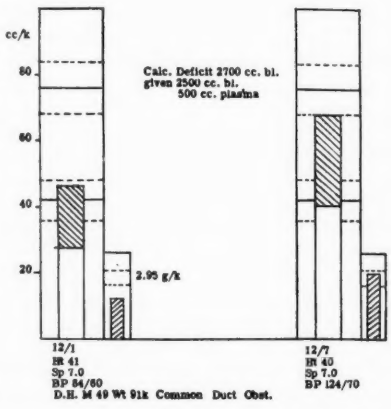

CHART 1. Case I—Acute blood loss uncompensated.

The column in each diagram represents total blood volume, the cross-hatched portion being the red blood cell mass, and the lower clear part the plasma volume—all in cc./kg. of body weight. The solid, horizontal line just above "40" represents the mean normal plasma volume, the broken lines above and below indicating the standard deviation of the mean. Similarly, the solid line below "80" is the mean normal total blood volume, again the dotted lines being the standard deviation of that mean. The small, cross-hatched column beside the main one is the protein value in gm./kg., and the dotted lines are normal limits, with 2.95 the mean normal value. This notation applies to all charts.

This case is typical of acute blood loss where homeostatic mechanisms have failed to restore the blood volume by hemodilution from the interstitial fluids. Gregersen[3] has stated that a 30% reduction in total blood volume is followed in a few hours by the development of shock unless compensation occurs. This is well demonstrated in the present case. Estimation of total blood loss and adequate and prompt replacement can prevent the development of shock in similar instances of acute blood loss.

Case II. H. S., a 65-year-old white male, was admitted to the hospital after he had vomited coffee ground material and fainted. He had had a gastroenterostomy for peptic ulcer 20 years previously. Recently he had had return of gastric symptoms and had tarry stools on a few occasions during the past few months. On admission his BP was 72/50. His hematocrit was 40. During the next 12 hours the patient received 2000 cc. of whole blood in spite of which the hematocrit fell to 27. The patient at no time was in profound shock, his blood pressures varied between 74/60 and 110/72. Three days after admission a blood volume was done (Chart 2). The findings showed a high normal total blood volume with a red blood cell deficit of 750 cc. The total circulating protein was normal although the protein concentration was 5.3 gm. %. The red blood cell deficit had been compensated for by an increase in plasma volume of 1100

cc. in excess of normal. Accordingly, the red blood cell deficit was replaced with blood from which the plasma had been removed and replaced with saline. The 750 cc. of red blood cells were administered over a 16-hour period. No further evidence of bleeding occurred and no additional intravenous therapy was given. Nine days later repeat blood volume showed essentially normal red blood cell volume with still some excess of plasma volume.

In this case blood loss was compensated for by hemodilution. Replacement with red blood cells suspended in saline was used to reduce the hazard of producing hypervolemia from the administration of whole blood.

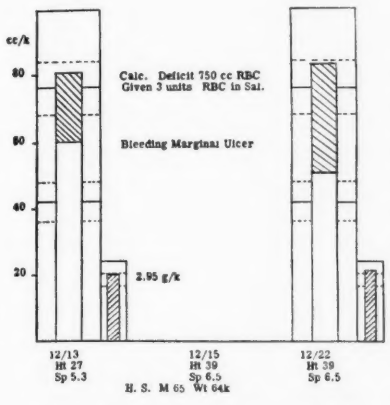

CHART 2. Case II—Acute blood loss compensated.

The question of the use of red blood cells suspended in saline or glucose solutions instead of whole blood in such instances of compensated blood loss has been of particular interest, especially where large red blood cell deficits are present and patients are in the older age groups. The influence of plasma protein of whole blood in producing a hypervolemia in such instances seemed worth exploring. Two cases with moderately severe anemias were studied with this point in mind. The case histories are as follows:

Case III. L. B. was a 56-year-old white male with diabetes and gangrene of his leg. The patient had already had an amputation of his leg when the blood volume was done. The patient needed 1200 cc. of red blood cells and was given 5 pints of whole blood in the next five days after which a second blood volume was done (Chart 3).

Case IV. P. was a 71-year-old white male who also had diabetes with gangrene of his extremity. This patient had an amputation before the blood volume was done. It showed a deficit of 800 cc. of red blood cells. Replacement was made with red blood cells suspended in saline (Chart 3). A repeat blood volume was done five days later.

Both cases showed severe anemia with normal plasma volumes and high total serum proteins. The calculated red blood cell deficits were quantitatively replaced in both instances. It is of interest to note the trend to hypervolemia in the patient receiving whole blood in contrast to the second patient who maintained both a normal total blood volume and plasma volume when red blood cells suspended in saline were used.

Red blood cells suspended in saline have been administered in a large number of similar cases and no instances of cardiac embarrassment or other evidences of hypervolemia have been encountered.

The most significant group of cases where blood volumes have proved helpful has been in patients with chronic disease accompanied by weight loss. It has been shown by a number of investigators[2] that patients with chronic

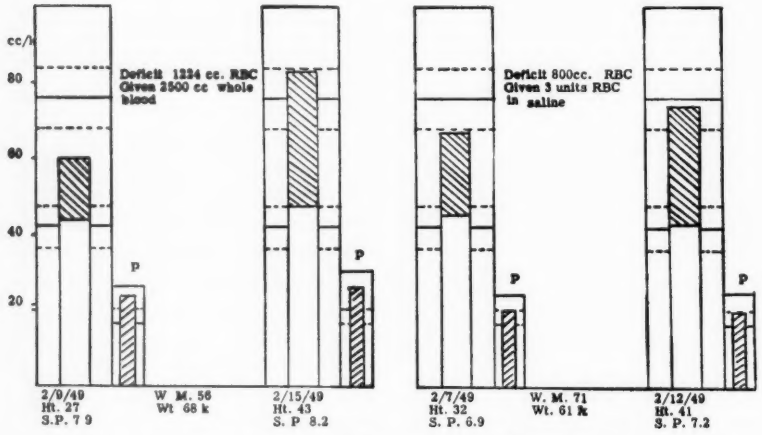

CHART 3. Cases III & IV—Comparison of replacement with whole blood versus RBC in saline.

wasting disease present a syndrome of weight loss, reduced blood volume, and increased interstitial fluid volume. The significant feature of these cases is an increased susceptibility to postoperative shock which is preventable by preoperative replacement of the blood deficit. The loss of weight in such individuals is attended by a depletion of tissue protein which is also reflected in a reduction of the serum proteins. The reduced oncotic pressure of the plasma together with the reduction of the tissue tension, as postulated by Schade, disturbs the normal 1:3 ratio of plasma volume to interstitial fluid volume and more fluid enters the enlarged tissue spaces from the vascular compartment. If anemia is present, it may be completely masked by this tendency to hemoconcentration. Hematocrits and serum proteins will give no indication of the large deficits which in many instances may be present. These findings are well demonstrated in the next case.

Case V. R. R. is a 27-year-old white male who has had chronic ulcerative colitis since 1943. It was quiescent until February, 1948, when he entered the hospital because of bloody diarrhea and weight loss of 20 kilos. He was treated medically for a month without improvement. During this period he received six transfusions. Surgical intervention was considered necessary and the patient was prepared for ileostomy. At this time the first of a series of blood volumes was done (Chart 4). This showed a deficit of 1200 cc. of red blood cells and also low serum proteins. The patient received 2500 cc. of whole blood before operation and 500 cc. during the procedure. He did well after the ileostomy, and a repeat blood volume 12 days later showed a quantitative replacement of red blood cells and slightly reduced total volume. The patient was discharged from the hospital but at home had a rapid return of his symptoms and he

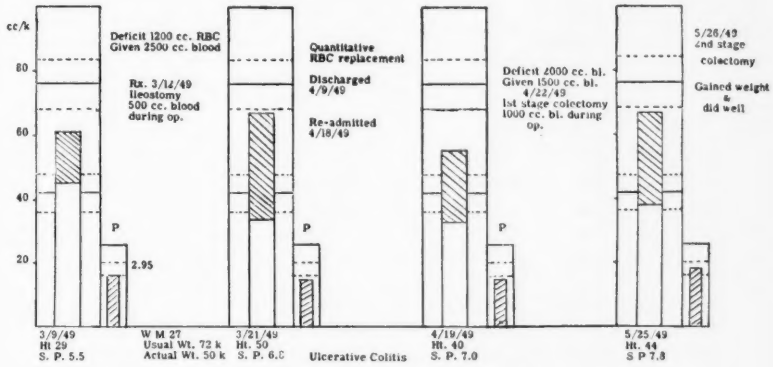

CHART 4. Case V—Chronic shock.

returned to the hospital five weeks after the ileostomy for the first stage of a total colectomy. The patient's general condition was poor and he still showed marked weight loss. Hematocrit at this time was 40 and the serum protein was 7.0 gm.%. The third blood volume showed, however, a 27% reduction in the total volume which affected the cells and plasma to the same degree and accounted for the normal hematocrit and protein values which were obtained. The deficit of 2000 cc. of blood was corrected with 1500 cc. of blood before operation and 1000 cc. during the operative procedure which was done three days after the blood volume determination. The patient withstood the operative procedure well. Postoperatively he showed marked improvement and gained 10 lbs. during the next month in the hospital. Approximately one month after the colectomy a repeat blood volume showed essentially normal status, although he had not received any further blood. At this time the second stage of the colectomy was done and following this procedure he continued to gain weight and do well.

This case, again, indicates a quantitative replacement of red blood cell deficits but more strikingly shows how misleading hematocrits and serum

proteins may be in patients who present this picture which Clark[2] has labeled as "chronic shock."

It is well established that in the presence of both a deficit of hemoglobin and tissue protein the rebuilding of hemoglobin takes priority over the rebuilding of tissue protein. The correction of the anemia in this patient permitted rebuilding of tissue protein, and this is reflected in his rapid gain in weight after the operative procedures.

The patients so far discussed have had conditions in which deficits in blood volume have been present. Several cases have been studied in which the hematocrits have indicated an excess of red blood cells. Blood volume determinations have proved of great assistance in differentiating the situation of relative polycythemia from absolute polycythemia, whether of the secondary or of the vera type. The following cases are examples.

Case VI. B. A. was a 54-year-old white male who had complained of headaches, dyspnea, and anorexia for one year. He also had had hematuria on one occasion. The patient had a ruddy complexion. Blood pressure was 140/90. A palpable mass was present in the left flank. Pyelograms showed a tumor in the left kidney. The hematocrit was 66. A diagnosis of polycythemia vera and kidney tumor was made. The blood volume determination showed an excess of 3000 cc. of red blood cells. He was bled 3500 cc. in 7 days with the hematocrit falling to 52. A left nephrectomy was done and the patient made an uneventful recovery (Chart 5).

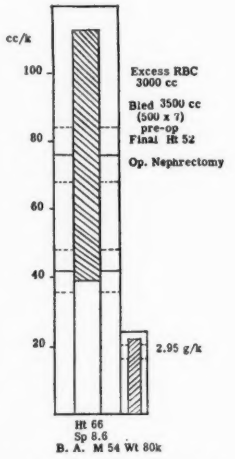

CHART 5. Case VI—Polycythemia vera.

The blood volume showed a red blood cell excess of 118% in contrast to the hematocrit which indicated only a 47% elevation in red cells. In similar instances where surgery is contemplated the blood volume gives a better evaluation of the situation; correction of the excesses, which is essential before major surgery is attempted, can be rapidly carried out.

Case VII. M. W. was a 64-year-old white female who had had a subtotal gastrectomy for carcinoma of the stomach 1½ years previously. In June of 1949 she had a return of gastric symptoms. Examination at the time of entry into the hospital showed an emaciated pale female who had moderate arteriosclerosis. Gastro-intestinal X-rays showed recurrent carcinoma of the stomach with obstruction of the jejunal loop of the enterostomy. The patient was operated upon on October 12, 1949, and had a resection of the remaining stomach, transverse colon, and spleen with an anastomosis of esophagus to the jejunum. The procedure required nine hours. The patient received

1500 cc. of blood preoperatively, 3500 cc. during the operation, and 500 cc. in the recovery room, a total of 5500 cc. The day after the operation auricular fibrillation developed and her blood pressure fell to 60/50. There were no signs of congestive heart failure present. An hematocrit was 55. Blood volume performed at this time (Chart 6) showed an excess of 970 cc. of red blood cells. A phlebotomy with removal of 500 cc. of blood was followed by a rise in the blood pressure and improvement in the patient's condition. Repeat blood volume the next day still showed 690 cc. excess of red cells and a second phlebotomy was done with 400 cc. of blood withdrawn. The

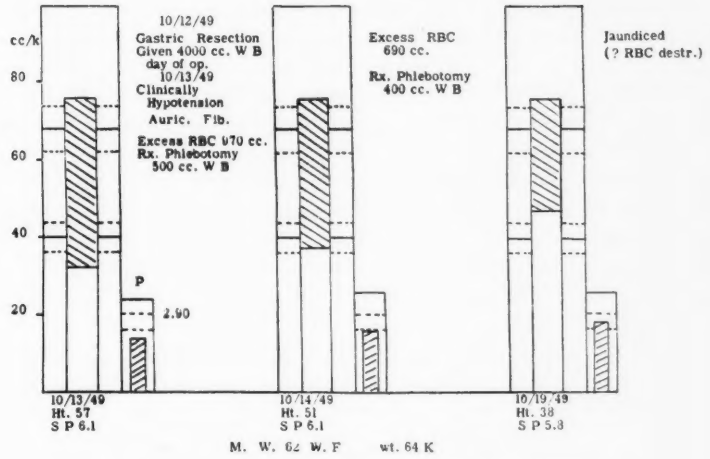

CHART 6. Case VII—Post-transfusion hypervolemia.

patient fainted during this procedure and no more blood was removed. The patient made an excellent recovery from this time on. A third volume study five days later showed a further fall in red cell volume to an essentially normal value although no further phlebotomies were performed. Concomitant with this fall, the patient became mildly jaundiced. She was discharged from the hospital on October 26, 1949.

It is apparent that this patient's vascular collapse was associated with the hypervolemia produced by the vigorous intravenous therapy during the operative procedure. The differentiation of simple hemoconcentration (relative polycythemia) from the absolute polycythemia which was present was an important factor in her recovery, for phlebotomy was done with prompt return of the blood pressure to normal levels. Had further intravenous therapy been given, cardiac embarrassment might have become more severe and might have led to a fatality.

Discussion

The present cases indicate some of the more outstanding problems which have been encountered in this study. It should be emphasized that such studies are especially helpful in the large group of patients with chronic disease such as malignancy where operative procedures are planned. Correction of deficits before operation has proved extremely important in reducing complications during operation and in speeding convalescence. The failure of hematocrits to indicate these deficits is well illustrated in a group of 44 patients with carcinoma of the colon who had blood volume studies done prior to operation. In this group there were 18 cases that had hematocrits of 40 or better. Of these, 11, or 60%, showed volume deficits of 2 or more pints. This deficit was due largely to a reduction of red blood cells which was completely masked by the hemoconcentration which was present.

It is worth noting that in the calculation of deficits for individuals who have had marked weight loss we have used the patient's best weight before his illness or his ideal weight if he has previously been obese. This has been recommended by Clark.[2] No evidence of overloading of the circulation has been encountered, although many cases have had deficits calculated on the basis of a weight 20-40 pounds greater than the actual weight at the time of the determination.

The Evans Blue method has proved a simple, reproducible clinical tool which can readily be set up in the average general hospital possessing ordinary laboratory facilities.

Summary and conclusions

1. Blood volume determinations are a valuable aid in the diagnosis and treatment of a variety of diseases, prominent among which are acute and chronic blood loss, dehydration, shock from whatever cause, malignancy with wasting, and chronic wound infections.

2. The Evans Blue method, as a clinical procedure, has proved practical for the average hospital. Each laboratory should set up its own series of normal values for plasma volume, red blood cell volume, total blood volume, and quantity of plasma protein, and these normals should be used for the determination of deficits or excesses in patients.

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THE INFERTILITY PROBLEM

A Commentary and Review of Three Years, Clinic Experience*

HERBERT THOMS

The clinic for the investigation and treatment of infertility problems was established by the Department of Obstetrics and Gynecology of the Yale University School of Medicine in 1946. This was the result of a desire to further more scientific and efficient handling of infertility problems, to better coordinate activities in this field in a teaching program, and to stimulate research.

Human reproduction has been a major concern of man since antiquity. At the present time the widespread interest of physicians, geneticists, biologists, physiologists, and others is witnessed by an increasingly voluminous literature on the subject and by the creation of new special society and study groups devoted to this subject.

A long overdue change in the attitude of physicians toward the proper investigation of the male as a contributing factor in the problem has resulted in important additions to our knowledge of the problem. Authorities are largely in agreement that in one third to one half of barren unions the husband is responsible for the difficulty.

Childless marriages are more common than ordinarily realized. Census figures show that 15 per cent of American couples have no children, and it has been stated that two thirds of these childless couples—one out of ten of all couples—are childless in spite of their desire to raise a family. This statement has not only the support of leading specialists in this field, but statistical support in the detailed studies of Whelpton and Kiser† of 1,977 couples.

In commenting upon this aspect of the problem Rock and Loth† state: "Even if the marriage rate falls below the prewar rate, there will still be more than a million and a half new couples every year, and for at least 150,000 of them this problem of infertility will become a personal one. Most of them will not find medical help because they will not know that

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* From the Department of Obstetrics and Gynecology, Yale University School of Medicine.

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† See Rock, J. and Loth, D. *Voluntary parenthood,* New York, 1949, pp. 71-72.

‡ *Ibid.,* p. 72.

anything can be done for them. Others will be unable to afford it. Still others will be discouraged by the prospect of undergoing tests and treatments. And a few will be ashamed to seek help because they will think that their condition must reflect some sort of wickedness or weakness."

Unlike many other physician-patient relationships the infertility problem directly concerns two individuals. The emotional part of the marital relationship often plays a major role and not infrequently an undercurrent of antagonism is present, one partner blaming the other for the failure. We are mindful of these antagonisms and self-recriminations and therefore emphasize the importance of studying each couple as an entity. A detailed history of each partner is obtained at the time of the first clinic visit. Many patients have had previous treatment elsewhere and the prospect of a further series of investigations is often discouraging. Any previous investigations are carefully interpreted and if data are available from other sources, it is secured. The first visit includes complete physical examination and the usual laboratory procedures, emphasis being placed upon the findings of the pelvic examination. At the first visit also, the patient is instructed in the technique of recording basal temperatures, and appointments are made for the basal metabolism determination and semen analysis. Examinations at subsequent visits are directed toward problems which are outlined below.

In infertility study too much emphasis cannot be placed upon the necessity for paying attention to what may be considered to be minor factors. It is probable that no group of physicians sees the result of sex ignorance more than those investigating infertility problems. It is remarkable at times how simple the correction of sex maladjustment may be, yet at other times barriers may be so obscure that it is only with greatest difficulty that aid can be given.

As previously noted, in from one third to one half of all cases the fault lies with the male partner. As a matter of fact, this responsibility is often greater because the transmission of gonorrhea may bring about conditions commonly included as female factors. Any plan of procedure should have available the services of a urologist with a special interest and knowledge of the field; we have been fortunate in securing such cooperation. Among the chief male factors which may be listed are (1) constitutional—systemic diseases which may be responsible for deficient spermatogenesis and impotency; (2) endocrine—hypo- and hyperthyroidism, pituitary infantilism, obesity of the endocrinopathic type; (3) mental overwork. Men living lives of stress and tension often show seminal deficiency. Other male factors

may be listed as local conditions in sex organs which would include coital difficulties, developmental abnormalities, occlusion of genital canal due to disease.

The female factors in infertility may be likewise listed: (1) constitutional, which often operate to suppress both menstruation and ovulation; (2) endocrine, in which the glands most often concerned are the thyroid, anterior hypophysis, and ovary; (3) acquired pelvic disease, occlusion of genital canal, inflammatory exudates acting mechanically or by changes in chemical reaction; (4) congenital anomalies; (5) coital factors, dyspareunia, vaginismus, effluvium seminis. In addition to the above, Novak* also lists "multiple causes" and states that in the majority of cases a careful study will reveal a number of factors in either or both parties, and whether or not pregnancy is to occur seems to depend upon the summation of the fertility level of the two partners.

Space does not permit a further discussion of the usual methods of study. These include tubal insufflation, hysterosalpingography, basal metabolism determination, endometrial biopsy, basal body temperature studies, evaluation of cervical smears, hormone studies. Treatment may include sex education, correction of local disease states, endocrine therapy, correction of malpositions, and aided insemination.

The infertility clinic at Yale was started as a separate enterprise on April 12, 1946. Previous to this time, patients presenting infertility problems were treated in the outpatient department or seen privately by members of the department staff. From January 1, 1947, to January 1, 1948, 70 couples were enrolled and 206 office visits recorded; 7 pregnancies were reported during this time.

From January, 1948 to January 1, 1949, 60 couples were enrolled and 228 office visits recorded; 10 pregnancies were reported during that time. From January, 1949, to January 1, 1950, 173 couples were enrolled and 423 office visits recorded; 39 pregnancies were recorded during that time.

Thus, in the three-year period, 303 couples have been enrolled and 56 pregnancies have been recorded. This latter figure represents 18.7 per cent of the former, but does not represent a true picture of the success of our efforts because a certain number of couples enrolled are still under active study. We have reason to believe that these results may be considered satisfactory.

Something might be said about the approach to these problems by group

* Novak, E. *Textbook of gynecology*, 3d ed., Baltimore, 1948, pp. 617-622.

endeavor in a clinic as contrasted with that of private practice. There is reason to believe that individual handling of these problems offers advantages. One advantage in private practice is in the timing of visits which can be made very much more flexible. It is often necessary to do certain diagnostic procedures in relation to the menstrual cycle in individuals. It is difficult to fit such studies into the clinic schedule. Other advantages include a more direct physician-patient relationship.

The advantages of the clinic endeavor are:

(1) It offers to people in the lower income groups an opportunity for study, often prolonged, at a professional fee which they can afford;

(2) The personnel of the clinic, physicians, nurses, technicians working as a group, tends to accelerate the time necessary for some of the laboratory studies which are part of the routine investigation;

(3) The clinic serves an important teaching function for physicians undergoing graduate training who thus have an opportunity to assist the physicians who are directing the clinic activities;

(4) By means of clinical conferences the clinic activities are carried over into the teaching program of the medical students.

From what has been said about the function of the clinic in offering service to people in low income groups it should be obvious that it could not do this without financial support other than that obtained from professional fees. In our clinic such support has been given almost from the beginning by The Planned Parenthood League of Connecticut. It is a pleasure to acknowledge their continued interest in our endeavor. Their help and cooperation have by no means been limited to the funds which have been placed at our disposal.

Summary

1. The problems of infertility have been generally considered.

2. A three-year clinic experience has been noted in terms of numbers studied and the results of therapy.

3. The advantages of infertility study in private and clinic practice have been commented upon.

CIRCULATORY FACTORS IN THE PATHOGENESIS OF EXPERIMENTAL ARTERIOLAR NECROSIS*

L. L. WATERS AND G. I. DESUTO-NAGY†

If the renal arteries of normal dogs are ligated, azotemia associated with some degree of hypertension occurs, and death results within a few days. In the heart, gall-bladder, stomach, and intestinal tract, medial necrosis of arterioles may be found together with focal hemorrhages and necrosis of smooth or cardiac muscle. The vascular changes were described by Goldblatt[2] and have been observed many times in this and other laboratories in animals subjected to severe renal damage.[7] Anatomically they differ in no respect from the arteriolar changes often encountered in man in malignant hypertension.

Beyond the frequent association of these lesions with hypertension and renal failure, very little is known of their pathogenesis. The experiments to be reported bear on the role of hypertension and of venous circulatory factors in the pathogenesis of experimental arteriolar necrosis in dogs.

Experiments with adrenalin

A relatively simple demonstration of the relationship of hypertension to experimental vascular disease would be the occurrence of arteriolar changes in animals following injection of vaso-pressor substances. Beginning in 1903, adrenalin and other pressor amines were injected repeatedly into laboratory animals in an effort to produce acute or chronic arterial disease.[4] Aortic medial hemorrhage and necrosis followed by patchy fibrosis or calcification was the usual result in rabbits. In dogs, less definite and less consistent changes were observed. In addition to aortic lesions, Duff and his associates[1] in 1939 reported the occurrence of scattered arteriolar necroses in rabbits given repeated injections of tyramine. This type of experiment has been discontinued probably because the commonly observed aortic lesions correspond only to rare forms of arterial disease in man.

In the course of investigations in this laboratory on the vascular-

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† With the technical assistance of Edward Iannucci, Peter Integlia, and Felix Ferraiolo.

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TABLE 1

SUMMARY OF LESIONS IN DOGS RECEIVING ADRENALIN

Dog no.	*Wt. in kgs.*	*Dosage of adrenalin*	*Duration of experiment*	*Aorta and pul. artery*	*Coronary arteries*	*Myocardium*	*Visceral arteries*	*Kidneys*
473	15.9	2 cc. in 5 minutes	20 minutes D	H	AN	H	not examined	not examined
474	12.2	5 cc. in 45 minutes	2 days S	H MN	AN	H MN	AN MN	
477	12.4	3 cc. in 1 hour on each of 4 days	4 days S	H MN	AN (severe) AH	H MN	AN	
478	14.5	4 cc. in 1 hour on each of 3 days	4 days S	H	AN	H MN		
479	12.1	3 or 4 cc. in 1 hour on each of 4 days	6 days S	H	AN	H MN		
480	10.8	4 cc. in 30 minutes on each of 3 days	4 days S	H MN	AN (severe) AH	H MN (severe)		AN
488	9.1	4 cc. in 30 minutes on each of 4 days	6 days S	H MN				
489	13.6	4 cc. in 30 minutes on each of 4 days	6 days S	H MN	AN	H MN	AN	AN
519	18.1	8 cc. in 30 minutes on each of 3 days	4 days D	H MN	AN (severe)	AN	AN	

Dog no.	*Wt. in kgs.*	*Dosage of adrenalin*	*Duration of experiment*	*Aorta and pul. artery*	*Coronary arteries*	*Myocardium*	*Visceral arteries*	*Kidneys*
520	11.3	8 cc. in 30 minutes	24 hours D	H	AN (severe)	MN		
521	13.2	8 cc. in 30 minutes on each of 3 days	4 days S	H MN	AN (severe)	MN	AN	AN
523	14.5	8 cc. in 30 minutes on each of 2 days	2 days S	H	AN	MN		
524	11.8	8 cc. in 30 minutes	12 hours D	H	AN	MN	AN	
525	19.5	8 cc. in 30 minutes on each of 3 days	4 days S	H		MN		
542	10.0	8 cc. in 30 minutes on each of 2 days	2 days D	H AN	AN (severe)	MN (severe)	AN	
543	11.3	8 cc. in 30 minutes on each of 2 days	2 days S	H MN	AN	MN	AN	
548	10.9	10 cc. in 30 minutes	1 day S	H	AN	H MN		
570	8.9	8 cc. in 30 minutes on each of 4 days	7 days S	H	AN (severe) Scars, H	MN (severe) Scars		AH AN

KEY—Table 1. A—artery, D—died, H—hemorrhage, M—muscle, N—necrosis, S—sacrificed.

damaging effects of various substances, it was found that intravenous injections of N-amylamine resulted, in dogs, not only in aortic hemorrhage and necrosis, but also in necrosis of muscular coronary arteries.[6] Since the dogs with lesions of their coronary arteries had maintained, following injection, mean femoral arterial pressures of 220-280 mm. Hg for approximately 30 minutes, enough adrenalin was injected into dogs to duplicate the extent and duration of this rise.

Usually from 4 to 8 cc. of standard 1:1000 solution given intravenously in divided doses was required. This of course is an enormous amount of adrenalin. But it should be pointed out that the arterial blood pressure that results is not far above that occurring in severe hypertension in man, and the hypertensive episode is measured in minutes rather than in months or years. Further, only five of eighteen animals have died following this regimen.

Histological examination of the tissues of eighteen dogs at varying intervals after one or repeated hypertensive episodes induced by adrenalin, revealed widespread damage to smooth and cardiac muscle (Table 1). Focal necrosis and hemorrhage of the media of the thoracic aorta were consistently present (Fig. 1). These changes were most conspicuous at the origins of the great arterial branches. Similar hemorrhages and necroses were found in the main stem of the pulmonary artery. Segmental fibrinoid necrosis and hemorrhage of small coronary arteries and arterioles was present, most frequently in vessels of the right ventricle and septum (Figs. 2, 3, 4). Similar changes were frequently present in small arteries in the gall-bladder (Fig. 5), stomach (Fig. 6), and colon. After more prolonged courses of adrenalin an occasional arcuate or interlobular vessel in the kidney was the seat of focal necrosis or hemorrhage, but as a rule the kidney vessels were not involved. No necrosis of cerebral or retinal vessels has been found.

Depending on the stage and severity of damage, there might be fibrinoid necrosis of the arterial media only, or a panarteritis with abundant periarterial cellular exudate. With time—a week or ten days—the vessel healed by medial and perivascular fibrosis. Focal necroses and hemorrhages in the myocardium were often extensive. These healed, leaving myocardial and subendocardial scars. Of the eighteen dogs injected with adrenalin, fifteen had numerous vascular lesions as described; three, only minimal changes. Experiments designed to yield more chronic lesions are in progress.

Dogs receiving injections of adrenalin, in addition to a marked rise in blood pressure, exhibited (as would be expected) strong peripheral

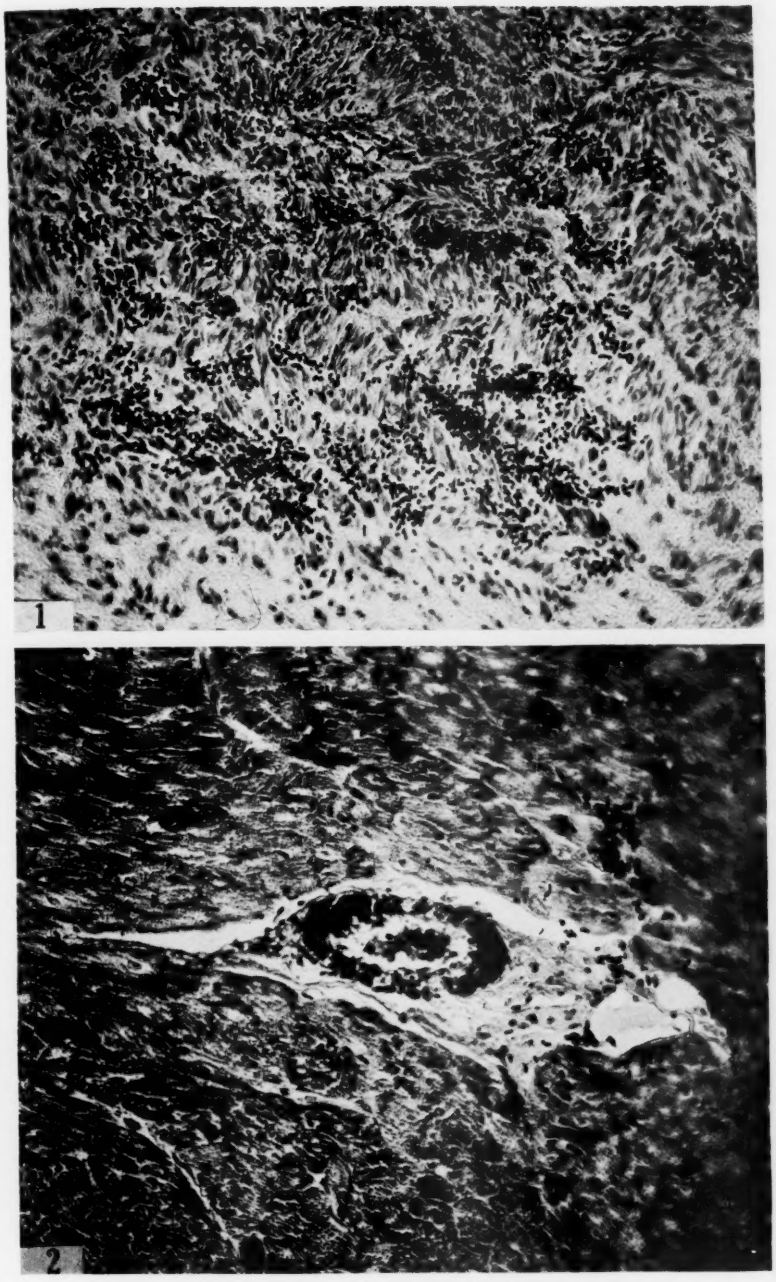
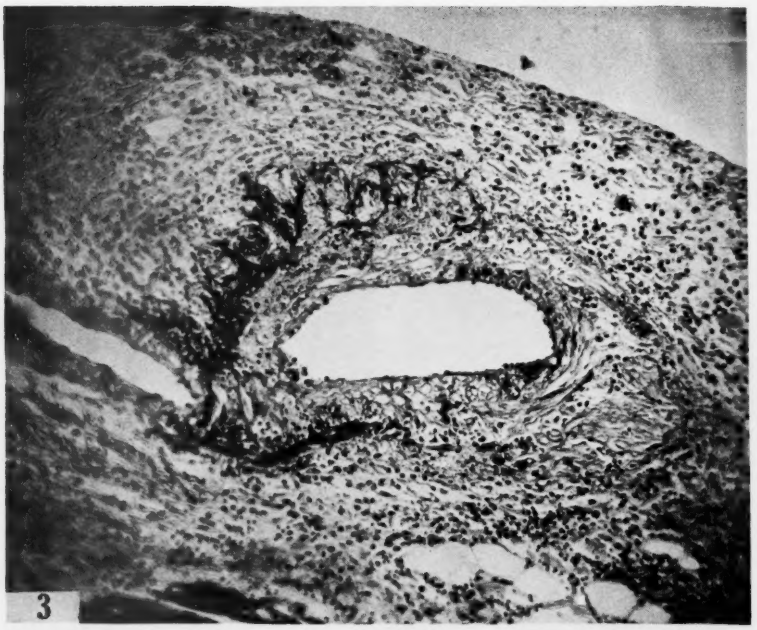


FIG. 1. Dog 477. Adrenalin. Hemorrhage in media of aorta. x 225.
FIG. 2. Dog 474. Adrenalin. Medial necrosis of coronary arteriole. x 275.



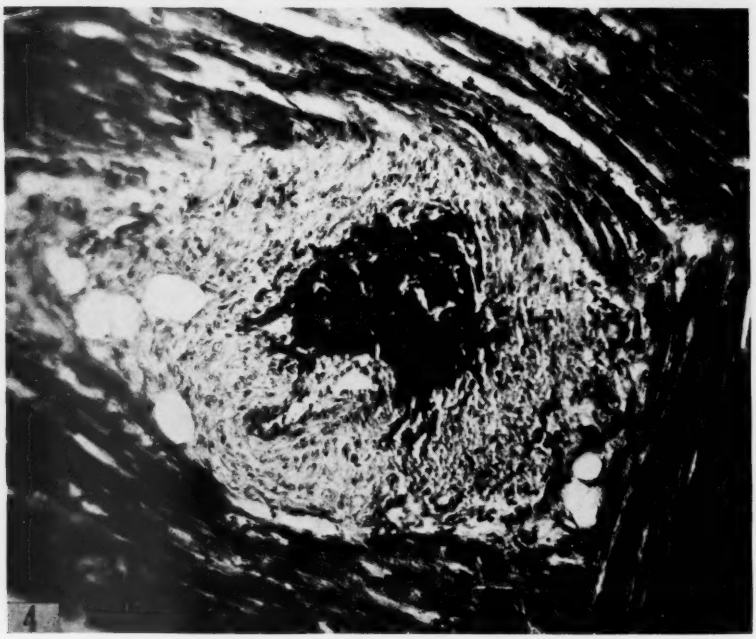
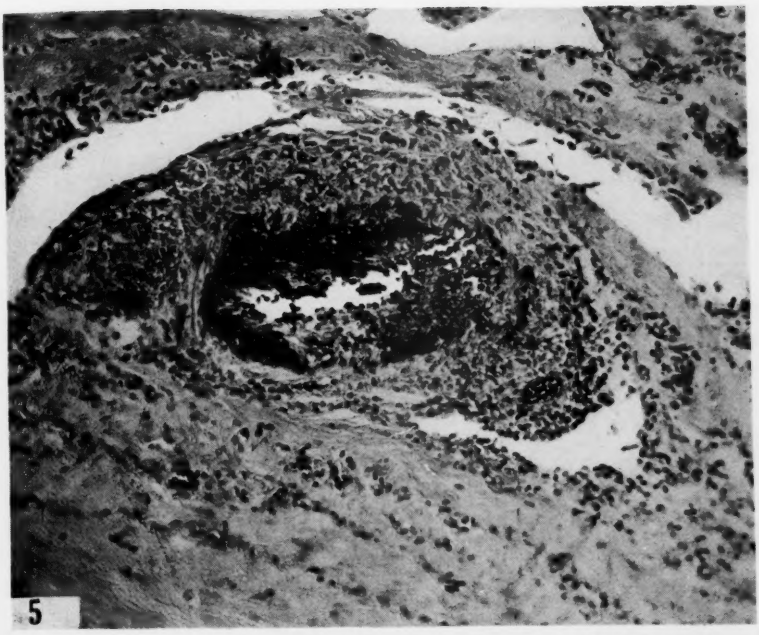


FIG. 3. Dog. 480. Adrenalin. Edema and necrosis of media of coronary artery. Periarterial cellular exudate. x 220.

FIG. 4. Dog 570. Adrenalin. Three days after last of four daily injections. Necrosis of media of coronary artery. Perivascular fibrosis. x 265.



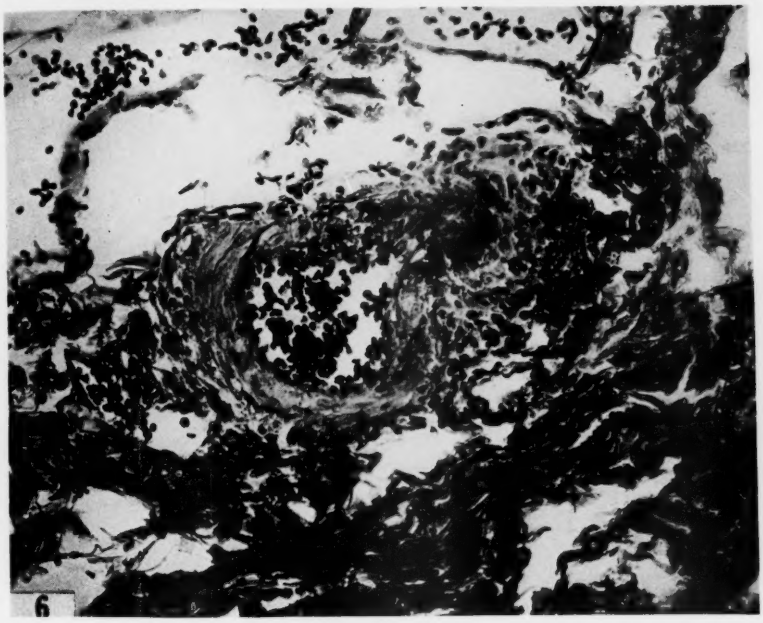


FIG. 5. Dog. 521. Adrenalin. Gall-bladder. Necrosis of arteriole in submucosa. x 250.
FIG. 6. Dog 474. Adrenalin. Stomach. Necrosis of branching submucosal arteriole. x 320.

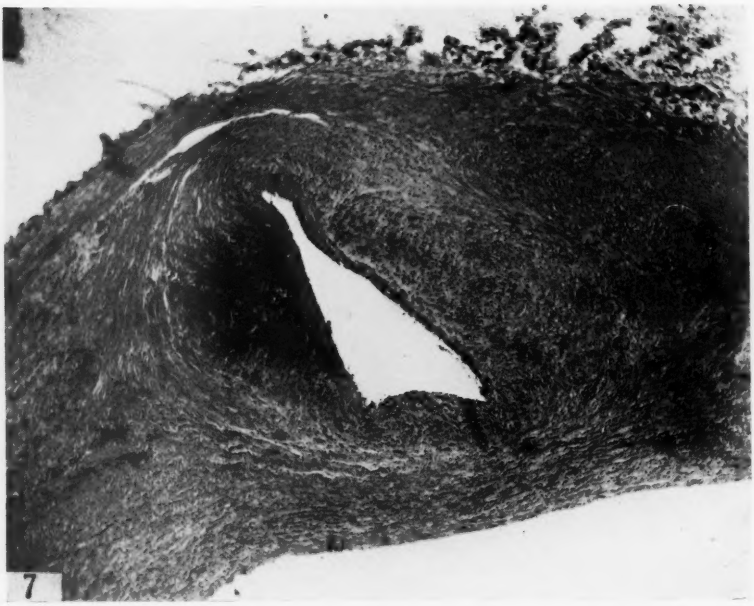
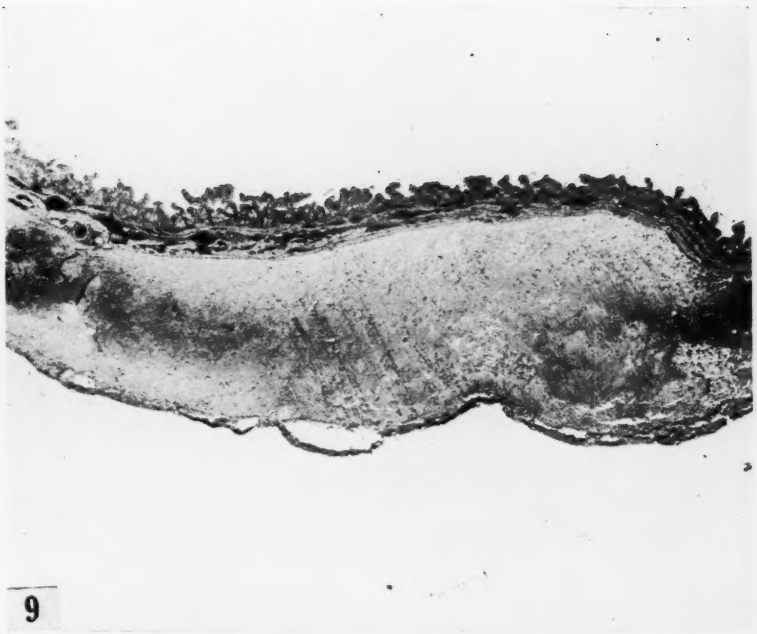


FIG. 7. Dog 597. Double nephrectomy. Transfusions of whole blood. Medial hemorrhages of aorta and innominate branch. x 40.

FIG. 8. Dog 608. Transfusions of whole blood. Necrosis of coronary arteriole. x 280.



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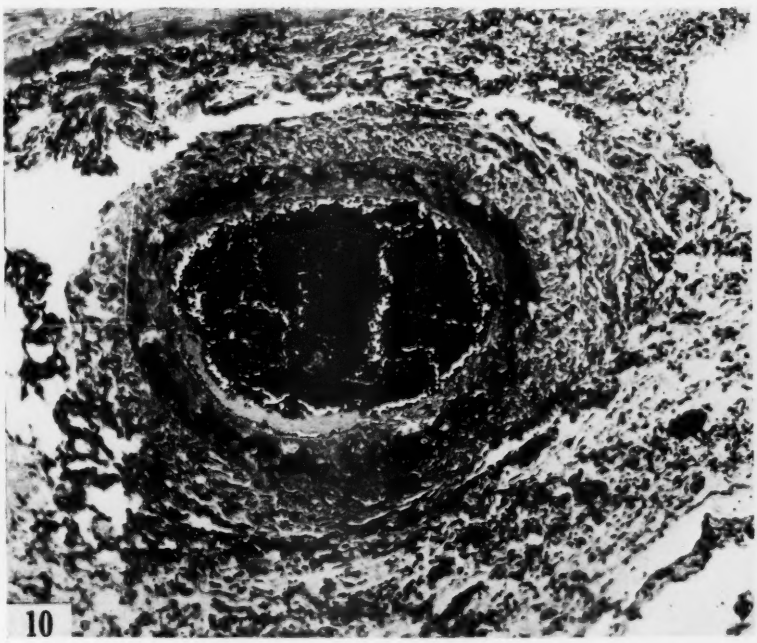

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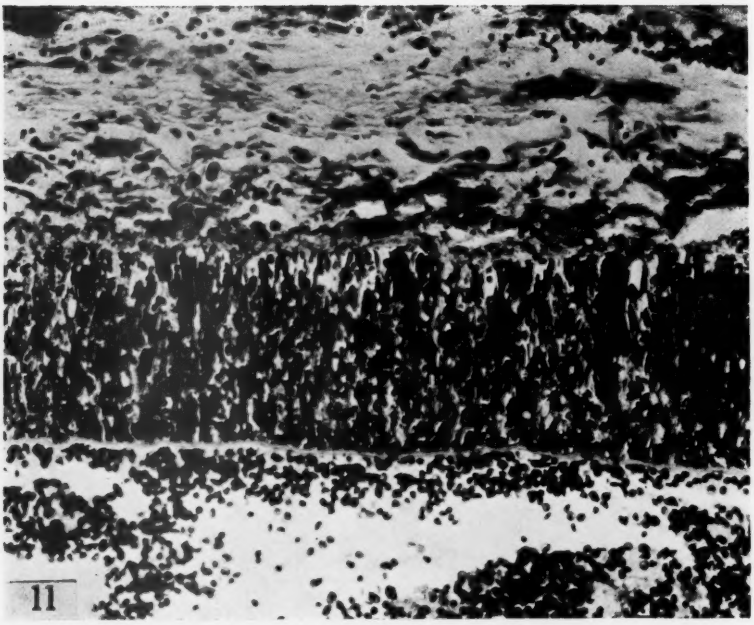
FIG. 9. Dog 516. Massive edema of gall-bladder one-half hour after a single large transfusion of blood. x 40.

FIG. 10. Dog 608. Transfusions of whole blood. Necrosis of artery in submucosa of gall-bladder. x 140.



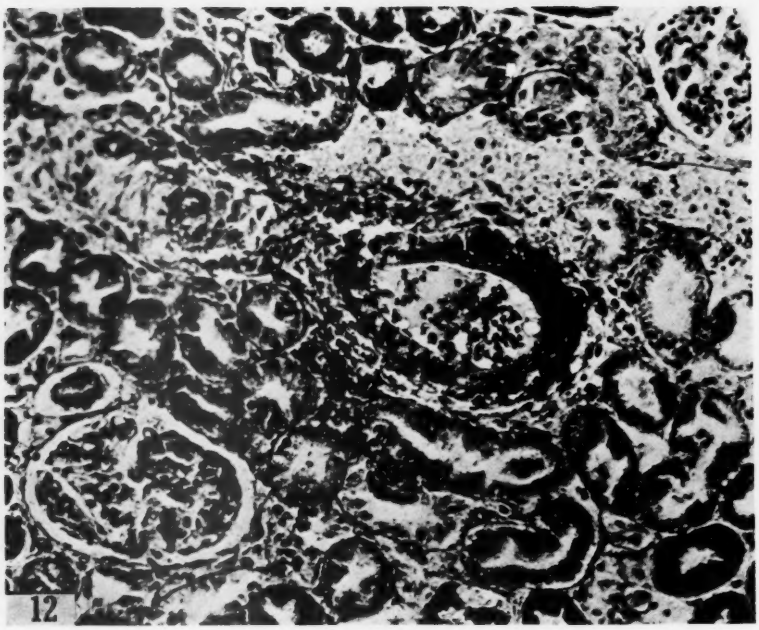


FIG. 11. Dog 572. Blood and adrenalin. Branch of renal artery. Medial edema, hemorrhage, and necrosis of smooth muscle. Loss of vascular endothelium. x 325.

FIG. 12. Dog 573. Blood and adrenalin. Kidney. Necrosis of interlobular artery. The glomeruli and cortical tubules are well preserved. x 300.

vasoconstriction. After some minutes of hypertension there was gross oxygen unsaturation of the arterial blood and air hunger as evidenced by moderate hyperpnoea. The cardiac rate was most often very rapid after the first injection, slow after succeeding ones. Direct observation of the heart during the injection period was carried out in a few animals not listed in the table. There was acute dilatation, particularly of the right ventricle and pulmonary conus. No gross narrowing of the superficial coronary arteries could be detected. The impression was gained that there was slight dilatation of these vessels. Dilatation of the coronary veins was prompt and maximal. None of the dogs receiving adrenalin developed azotemia.

Of interest, as will be brought out in a following section, was the direct observation that the gall-bladder, often after 1 to 2 cc. of adrenalin, became grossly edematous and developed ecchymotic spots. The liver was dusky and engorged. Because of these findings, jugular vein pressures were taken and were found to be greatly elevated, the rise paralleling that of the arterial pressure in duration (Tracing 1). Venous pressure values two and even three times the control readings were recorded. Once the administration of adrenalin was discontinued, the venous pressures fell to preinjection levels.

Since there are available a number of effective adrenolytic drugs, the preventative effect of one of these, N,N-dibenzyl-β-chloroethylamine, Dibenamine,* was tested. A series of seven dogs were pretreated with 20 mg./kg. Dibenamine[5] intravenously and subsequently were injected with large amounts of adrenalin. The blood pressure rise was, in each instance, completely abolished, but the cardiac acceleration due to adrenalin was retained. Subsequent histological examination of the tissues of these animals at appropriate intervals revealed no blood vessel changes whatsoever and in only one dog were minute foci of myocardial necrosis found.

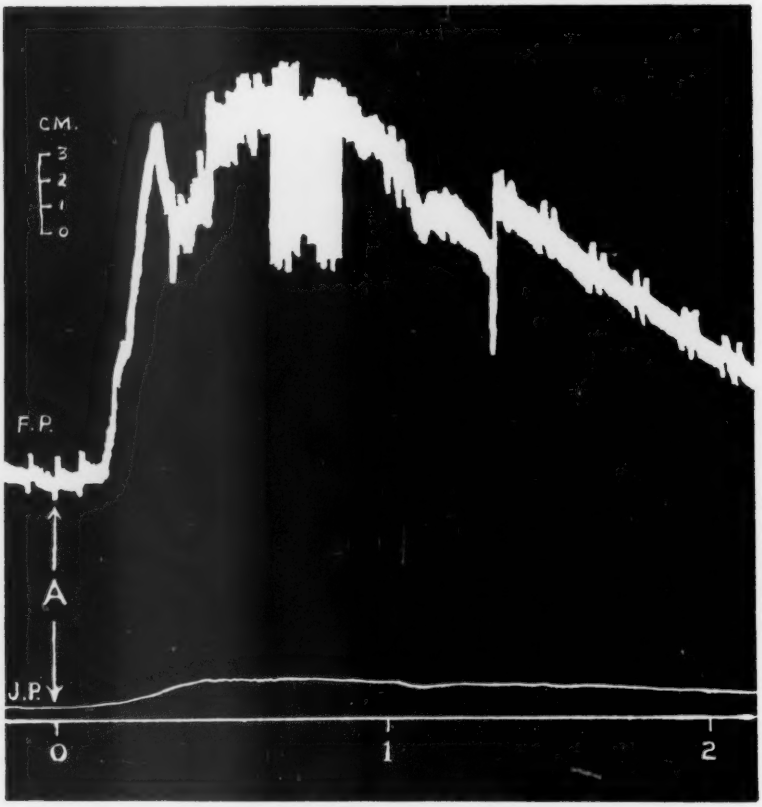
In the Dibenamine protected dogs, as already stated, there was no rise of arterial pressure with adrenalin; there was also no peripheral vasoconstriction or rise in venous pressure. Gross evidence of anoxemia was absent.

Experiments with blood

From the preceding observations with adrenalin it was clear that the arteriolar and cardiac changes following adrenalin administration were associated not only with the presence of systemic hypertension, but also with the presence of greatly increased venous pressure, with cardiac dilata-

* Courtesy of Smith, Kline and French, Inc.

tion, and with anoxemia. It was noted also that shortly after injection the right heart, liver, gall-bladder, stomach, and intestines became the seat of enormous venous congestion. It was of interest, therefore, to test the

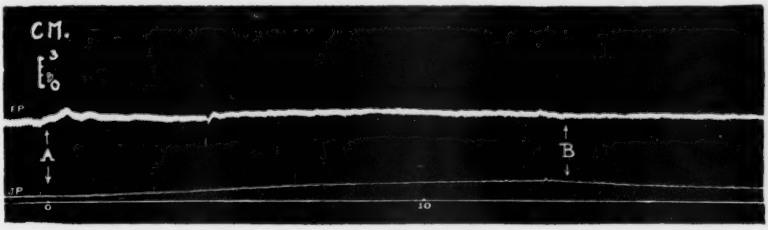


TRACING 1. Dog 585. Weight 9.5 kgs. Femoral arterial pressure (F.P.) and jugular venous pressure (J.P.) in cm. Hg after injection of 1 cc. 1:1000 adrenalin at A. Time in minutes. The venous pressure returned to preinjection level after 4 minutes.

vascular-damaging effects of increased venous pressure and filling without a factor of marked arterial constriction as contributed by adrenalin.

A series of eight normal dogs were given daily massive transfusions of citrated compatible dog blood in an effort to reproduce the rise in venous pressure, the acute

cardiac dilatation, and the anoxemia observed in animals receiving adrenalin. Blood was run in rapidly by vein until incipient cyanosis or severe vomiting occurred. This stage was usually reached after 50-100 cc./kg. of whole blood had been transfused. Venous pressures rose markedly (Tracing 2). During such massive transfusions a transient rise in arterial pressure of 15-30 mm. Hg was ordinarily observed. The mean femoral pressures never exceeded 170 mm. Hg in these dogs. The experimental animals received transfusions of this size daily for 3 to 8 days (Table 2). Gross and histologic examination of their tissues was carried out at intervals after the last transfusion.



TRACING 2. Dog 620. Weight 10.0 kgs. Femoral arterial pressure (F.P.) and jugular venous pressure (J.P.) in cm. Hg during and after rapid transfusion of 300 cc. compatible dog blood. Transfusion begun at A, terminated at B. Time in minutes. The venous pressure returned to preinjection level after 25 minutes.

The changes in the heart, gall-bladder, and gastro-intestinal tract after massive transfusions of citrated compatible dog blood reproduced many of those following injections of large amounts of adrenalin (Table 2, Figs. 7, 8, 9, 10). Extensive hemorrhages and areas of fresh necrosis were consistently present in the heart. Medial hemorrhage and necrosis of the aorta and pulmonary artery were found in four dogs. There was widespread fibrinoid necrosis of the small coronary arteries. Edema, hemorrhage, and necrosis of smooth muscle of the gall-bladder, stomach, and rectum were prominent. Widespread arteriolar necroses were present in these viscera. There was marked central congestion of the liver, sometimes with early necrosis. Branches of the hepatic artery were often necrotic. After massive infusions of blood the kidneys revealed numerous cortical petechial interstitial hemorrhages. Cloudy swelling of both proximal and distal tubules was present but not marked. There was no necrosis of tubular epithelium. The glomeruli were generally intact although their capillary tufts were edematous. An occasional arcuate or interlobular cortical artery was the seat of medial hemorrhage or early fibrinoid necrosis. These changes were more frequently observed in dogs receiving blood than in

TABLE 2

SUMMARY OF LESIONS IN DOGS RECEIVING BLOOD

Dog no.	*Wt. in kgs.*	*Treatment received*	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*	*Day 8*	*Aorta and pul. art.*	*Coronary arteries*	*Myo-cardium*	*Visceral arteries*	*Kidneys*	*Other*
552	5.0	Whole blood	475 cc.	500 cc.	450 cc.	S					H AN	AN (severe)	H AN	AN	H AN	Liver AN
561	5.0	Whole blood	500 cc.	440 cc.	400 cc.			S				AN	H MN	AN	AN	
564	8.6	Whole blood	425 cc. NPN 34.0	500 cc.	425 cc. NPN 49.0	300 cc. NPN 70.0	D					AN (severe)	H MN	AN	AN	
567	5.4	Whole blood	250 cc.	250 cc.	250 cc. NPN 42.0	NPN 43.0	NPN 53.2	NPN 39.2	S		H		H MN		H AN	
571	9.2	(Bilateral nephrecto-my) Whole blood	400 cc.	400 cc. NPN 156.1	400 cc. NPN 230.3	D					H AN	AN (severe)	MN			
576	5.4	Whole blood	350 cc. NPN 24.5	350 cc. NPN 25.2	350 cc. NPN 30.8	D						AN	H MN		AN	
577	6.8	Whole blood	330 cc. NPN 27.3	375 cc.	375 cc. NPN 30.8	350 cc. NPN 32.2	350 cc. NPN 44.1	350 cc. NPN 45.5	350 cc. NPN 63.0	350 cc. NPN 81.2 S		AN (severe)	H MN	AN	AN	Liver AN

Dog no.	*Wt. in kgs.*	*Treatment received*	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*	*Day 8*	*Aorta and pul. art.*	*Coronary arteries*	*Myo-cardium*	*Visceral arteries*	*Kidneys*	*Other*
582	6.3	Blood plasma	300 cc. NPN 37.8	300 cc. NPN 24.5	425 cc. NPN 29.0	400 cc. NPN 34.3	300 cc. NPN 30.8	375 cc. NPN 31.2	S			AN (severe)	MN H			
597	6.4	(Bilateral nephrecto-my) Whole blood			300 cc.	600 cc.		D			H AN	H AN (severe)	AN	H MN	H AN	
598	6.8	(Bilateral nephrecto-my) Whole blood			300 cc.	300 cc.	D						H MN			Liver H AN
608	10.0	Whole blood	350 cc. He=53 NPN 25.6	450 cc. He=55 NPN 29.4	550 cc. He=64 NPN 35.0	450 cc. He=68 NPN 48.0	S				H AN	AN (severe)	H AN	H AN	AN	Liver H AN
613	9.5	Whole blood	400 cc.	450 cc.	450 cc.	450 cc. He=70 NPN 33.0	450 cc. He=73	475 cc. NPN 44.2	S		H AN	AN	H	AN		

KEY—Table 2. A-artery, D-died, H-hemorrhage, M-muscle, N-necrosis, S-sacrificed, NPN-blood non-protein nitrogen in mgms. per 100 cc., He-hematocrit.

those receiving adrenalin alone. In two dogs hemoglobin was present in a few of the glomerular spaces and in distal convoluted tubules. In the other animals no hemoglobin was found in the kidneys. As already mentioned, tubular changes were not conspicuous.

Only preliminary physiological and chemical study of these animals has been made. None of the dogs receiving blood has had an arterial blood pressure above 170 mm. Hg during the course of the experiment. Hematocrits, as would be expected, became progressively elevated in the dogs receiving blood. Blood non-protein nitrogen was moderately elevated; urinary output increased. The animals receiving blood became ill, vomited often, and some died before termination. Six of the eight dogs receiving whole blood had widespread vascular and smooth muscle lesions; two, minimal lesions of this type.

Besides the group of normal dogs receiving blood, three dogs were given whole blood following double nephrectomy. Two of these developed many vascular changes, the third, minimal lesions (Table 2). One normal dog (No. 582) was given plasma instead of whole blood on six consecutive days. Severe necrosis of the coronary arterioles and myocardium was present in this animal.

Experiments with blood and adrenalin

A group of six dogs has been given a combination of large amounts of compatible blood followed immediately by an intravenous injection of 1 cc. of 1:1000 adrenalin solution. This procedure was carried out in each animal on several successive days. Extensive arteriolar necroses in the heart, gall-bladder, and gastro-intestinal tract have occurred in five of these dogs (Table 3).

The kidney findings in this group have been of particular interest. Grossly the organs are large, with pale cortices. Fleck-like interstitial hemorrhages are present beneath the capsule. On the cut surface the pale cortex contrasts with the enormously congested medulla. Numerous confluent petechial hemorrhages are present in the boundary zone. On microscopic examination, widespread fibrinoid necrosis and hemorrhage are found in the walls of the renal arteries and arterioles (Figs. 11 and 12). The arcuate and interlobular vessels are most extensively involved, with occasional necrosis of an afferent glomerular arteriole. The glomeruli themselves are intact except for some edema. There is no necrosis or even marked cloudy swelling of the cortical tubules. The boundary zone of the kidney is the seat of enormous venous congestion and interstitial hemorrhages.

TABLE 3

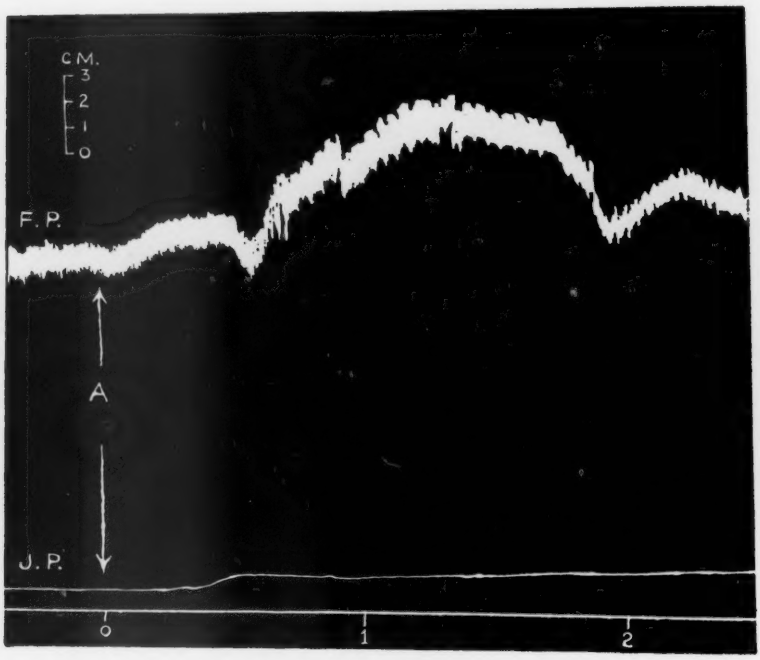
SUMMARY OF LESIONS IN DOGS RECEIVING BLOOD AND ADRENALIN

Dog no.	*Wt. in kgs.*	*Treatment received*	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*	*Day 8*	*Aorta and pul. art.*	*Coronary arteries*	*Myo-cardium*	*Visceral arteries*	*Kidneys*	*Other*
565	3.6	Blood and adrenalin	200 cc. and 1 cc.	200 cc. and 1 cc.	D Pneu-monia								H MN		H	Diffuse Pneumo-nia
569	4.5	Blood and adrenalin	175 cc. and 1 cc.	rest NPN 95.9	rest NPN 33.6	150 cc. and 1 cc. NPN 44.1	185 cc. and 1 cc. NPN 34.2	250 cc. and 1 cc NPN 38.5	250 cc. and 1 cc NPN 41.3	S	H AN	AN Fibro-sis (severe)	MN scars	AN	AN AH (severe)	
572	5.9	Blood and adrenalin	400 cc. and 1 cc.	350 cc. and 1 cc. NPN 47.6	350 cc. and 1 cc. NPN 57.4	D					H	AN (severe)	H MN	AN	AN (severe)	Liver AN Adrenal AN Lymph nodes AN
573	7.2	Blood and adrenalin	435 cc. and 1 cc.	300 cc. and 1 cc. NPN 53.8	400 cc. and 1 cc. NPN 39.2	350 cc. and 1 cc. NPN 46.9	S NPN 69.7				H AN	AN (severe)	MN H	AN	AN (severe)	Liver AN
618	5.4	Blood and adrenalin	300 cc. and 1 cc. NPN 27.2	300 cc. and 1 cc.	300 cc. and 1 cc.	300 cc. and 1 cc.	NPN 25.0	S				AN	MN H	AN	AN (severe)	Liver AN
619	7.2	Blood and adrenalin	350 cc. and 1 cc. NPN 34.5	350 cc. and 2 cc.	420 cc. and 1 cc.	350 cc. and 1 cc.	350 cc. and 1 cc NPN 52.4	S			H AN	AN (severe)	MN H	AN	AN (severe)	Liver AN Spleen AN Adrenal AN

KEY—Table 3. A-artery, D-died, H-hemorrhage, M-muscle, N-necrosis, S-sacrificed, NPN-blood non-protein nitrogen in mgms. per 100 cc.

Experiments with renal extracts

Since a pattern of cardiovascular damage similar to that seen in experimental renal insufficiency occurs after adrenalin administration or the transfusion of large quantities of blood, it was of immediate interest to

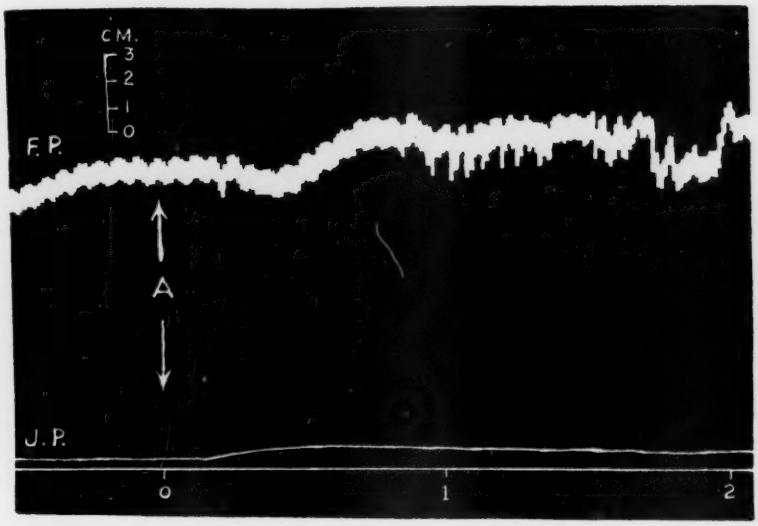


TRACING 3. Dog 589. Weight 9.1 kgs. Femoral arterial pressure (F.P.) and jugular venous pressure (J.P.) in cm. Hg after intravenous injection of 25 cc. vasopressor renal extract at A. Time in minutes. The arterial and venous pressures returned to preinjection levels after 18 minutes.

see if similar circulatory changes are present in conditions of experimental renal insufficiency that result in arteriolar necrosis.

The injection of pressor extracts of normal hog kidney into doubly nephrectomized dogs usually results in widespread arteriolar necroses.[7c] Four dogs were therefore nephrectomized and pressor extracts of hog kidney were prepared according to a known procedure.[7c] On the fourth day following nephrectomy, under light pentothal anesthesia, the animals were

given two intravenous injections of 25 cc. of kidney extract 20 minutes apart. Femoral arterial and jugular venous pressures were recorded and a small incision was made over the costal margin in the mid-line so that the gall-bladder could be observed. Injections of the extract resulted in arterial pressor responses of the renin type. Venous pressure values rose abruptly in some instances to at least double the pre-injection level (Tracing 3).



TRACING 4. Dog 590. Weight 4.5 kgs. Femoral arterial pressure (F.P.) and jugular venous pressure (J.P.) in cm. Hg after intravenous injection of 25 cc. vasopressor renal extract at A. Time in minutes. Small rise in arterial pressure. Arterial and venous pressures returned to preinjection levels after 14 minutes.

The duration of the venous pressure rise followed the curve of the arterial pressure. It was noted in some instances that although there was very little total rise in arterial pressure, the rise in venous pressure was large (Tracing 4).

During and following the injection of renal extract the gall-bladder was observed from time to time. At the beginning of the experiment this organ was thin-walled and a translucent blue-green in color. Within a few minutes a gross alteration occurred. Starting at the base, the gall-bladder wall became thick, white, and opaque. This change spread towards the fundus, so that within 30 minutes the whole sac was edematous and distended.

Petechial hemorrhages appeared beneath the serosa, and larger hemorrhages deeper in the wall. The gross appearance of the gall-bladder was similar to that occurring frequently after adrenalin administration, or after the infusion of large quantities of blood. In animals sacrificed 30 minutes to one hour after injections of renal extract as described, not only was the edematous, hemorrhagic gall-bladder found, but also fresh myocardial and pulmonary artery hemorrhages similar in location to those seen after injections of adrenalin, blood, or bilateral ligation of the renal arteries. There is, of course, ample evidence, previously obtained,[7c] that repeated injections of renal extracts into nephrectomized dogs result in fibrinoid necrosis of the arterioles of the heart and viscera.

Discussion

The first section of this paper describes the occurrence of widespread aortic, arteriolar, and myocardial lesions in dogs after large intravenous injections of adrenalin. That the vasoconstriction and rise in arterial pressure are factors in the pathogenesis of these changes is suggested by the fact that the lesions can be prevented by pretreatment with Dibenamine, which selectively abolishes the pressor effects of adrenalin. In the acute experiments described, no factor of gross renal failure was present. Nevertheless, the type of arteriolar and smooth muscle lesions and their distribution are similar to those that follow experimentally produced renal damage.

In addition to relating arteriolar necroses to vasoconstriction and hypertension, the experiments with adrenalin reveal that in this instance there are also other circulatory phenomena, namely, venous pressure and filling, cardiac dilatation, and anoxemia to be considered. The significance of these factors becomes apparent in subsequent experiments involving transfusion of blood.

Injection of adrenalin into dogs provides a relatively simple experimental method for the production of acute arteriolar lesions for anatomical and biochemical study. Investigation of the relation of such acute lesions to arteriolosclerosis is in progress.

The myocardial necroses that follow adrenalin injections afford material for a correlative study of anatomical and biochemical changes in the myocardium with reproduceable and measurable changes in the peripheral circulation.

Finally, the demonstration that a chemotherapeutic agent, Dibenamine, can almost completely prevent the anatomical changes associated with adrenalin injection should intensify search for chemotherapeutic substances effective prophylactically in hypertension in man.

Following the adrenalin experiments, the vascular-damaging effects of intravenous injections of large quantities of blood are described. The type and distribution of the lesions occurring after these injections are similar to those occurring in experimental renal insufficiency, or following adrenalin injection. These experiments demonstrate further that extreme hypertension is not essential to the development of widespread experimental arteriolar necrosis. They emphasize again that factors of blood volume, hemo-concentration, venous pressure, local venous filling, cardiac dilatation, and anoxemia may be of importance. It is interesting that Holden and his associates[3] have recently and independently demonstrated the deleterious effect on the hearts of dogs of massive transfusions of blood. Because of the renal damage demonstrated in dogs receiving massive blood transfusions, humoral factors of renal origin exerting a direct toxic effect on blood vessel walls may be implicated as etiologic factors in the arterial changes observed. However, nephrectomized dogs given blood develop identical vascular lesions. Sensitization to foreign proteins as a factor in the etiology of the cardiovascular lesions occurring after massive blood transfusions is not ruled out by the experiments reported. The lack of gross signs of anaphylaxis, the early appearance of the changes, and the massive quantities of blood necessary to produce them argue against this possibility.

Next described are experiments combining blood transfusions with adrenalin injection. These demonstrate the synergistic effect of these procedures in producing lesions of arterial and cardiac muscle. In dogs receiving blood, only 1 cc. of adrenalin, per dose, instead of four or eight, resulted in severe vascular damage. The finding of widespread arterial changes in the kidneys of the dogs of this group may allow the experimental reproduction of some of the anatomical changes occurring in nephrosclerosis in man.

The most recent experiments demonstrate that pressor fractions of kidney protein injected into nephrectomized dogs result not only in increased arterial pressure but also in markedly elevated venous pressures and in congestive phenomena similar to those observed after injections of adrenalin or large blood transfusions. These findings suggest the possibility that mechanical circulatory factors may be active in the production of arteriolar necroses associated with experimental renal disease.

Summary

The same arteriolar lesions and similar changes in venous circulatory dynamics are described in three types of experimental procedures, each of

which leads to arteriolar necroses in the dog. The experimental procedures include: (1) injections of large amounts of adrenalin, (2) rapid intravenous injection of large volumes of blood, or blood and adrenalin, and (3) injections of extracts of pressor renal proteins into nephrectomized dogs. It is suggested that changes in venous pressure, blood volume, hematocrit, and oxygen saturation of the blood may play a role in the pathogenesis of the arteriolar lesions observed. Arterial constriction, in addition to any direct vascular damaging action it may have, may also act indirectly on the vessel walls by initiating physiological changes in the venous circulation.

The arteriolar lesions that follow injections of large quantities of adrenalin can be prevented by pretreatment with an adrenolytic substance, Dibenamine.

Injections of adrenalin combined with massive transfusions of blood result not only in arterial damage in the heart, aorta, and gastro-intestinal tract, but also in diffuse arterial necrosis and hemorrhage in the kidneys of intact dogs.

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A MEMORANDUM ON "WINTER" AT THE HOPKINS

GEORGE H. WHIPPLE

It is a great pleasure to set down here some notes about my contacts with Dr. Milton Winternitz many years ago and thus to participate in a very small way in a volume being prepared by his friends to do him honor in the year of his retirement.

My contacts with "Winter" are all early and go back to his graduation in medicine at Johns Hopkins in 1907. Winter came into the Department of Pathology his graduation year as I had done after my graduation in 1905. We were very young and tried our teeth on everything medical, pathological or not. Both of us had ideas which sometimes were divergent and at times the sparks flew. As did all members of the department, we had great admiration and respect for William B. MacCallum and Dr. William H. Welch. We watched them and tried to learn from them, even if our contacts with Dr. Welch were not frequent. Winter at this time was working in the field of enzymes (in particular, catalase and lipase).

We shared the autopsy routine and helped in the medical student teaching. Later, when MacCallum went to Columbia, we shared much of the lecturing for the second-year class. We often laid plots to catch Dr. Welch for lectures in pathology as we enjoyed them as much or if possible even more than the students. Sometimes our plots failed and Dr. Welch slipped away leaving us to handle the lecture as best we might. After a few experiences of being caught short we laid out our lecture responsibility—one or the other of us being prepared to lecture whether Dr. Welch turned up or not. It was a valuable experience to prepare a lecture on a given topic and have the pleasure of measuring our lecture (not given) with that which was given by Dr. Welch. This caused no inflation of the ego.

We also gave clinical pathological conferences with Dr. Thayer and others. Winter often had tilts with the clinical groups, his words at time sharp but with humor. The students learned much from him; they were never bored, never asleep, and they admired him as a teacher.

Winter is a crusader at heart. He loves the good fight and sometimes perhaps is believed to get into an argument because the smoke of battle is always stimulating and pleasant to him. Medicine needs crusaders especially today; there are too few of them. That is one of many reasons why Winter's friends mourn his retirement and rejoice in his past accomplishments.

"WINTER" AT YALE

C.-E. A. WINSLOW

The history of the Yale School of Medicine is illuminated by the names of many great men. A distinguished school is a living organism and the soul which inspires and guides it has been created by the dreams and the visions which have gone before. In the Yale School of Medicine, no one of our distinguished leaders has played a greater role in this creative process than Milton Winternitz.

When "Winter" became our Dean, thirty-three years ago, there were but five men of professorial rank in the School (one of whom was resigning at the end of the year) with two professors from other departments of the University also giving courses to our students. There was but one modern building, the rest of the instruction being carried on in old and badly equipped structures scattered over the Yale campus. There were nineteen students in the graduating class of 1920. During the war years, there was a very popular pool in one department in which the faculty placed their bets on one of three future alternatives: continuation of a four-year school, reduction to a two-year school or entire abandonment of medical education at Yale.

In 1922, two years after he became Dean, Winternitz prepared a report on the "Past, Present and Future of the Yale University School of Medicine" which has been described as "one of the remarkable documents in the history of medical education." This report visualized the complete reconstruction and centralization of the physical plant of School and Hospital; it laid the groundwork for raising the quality of the faculty and student body to the highest levels; and it envisioned the establishment of a School of Nursing on a true university basis.

The ideals which should animate the new medical section of the University were outlined, essentially as follows:

1. Medicine is a progressive science which can advance only in the spirit of the investigator, to be exercised at the bedside as well as in the laboratory.

2. Medicine should deal with the welfare of the patient through his whole life and should visualize the promotion of health as a positive ideal. It should not concern itself merely with the diagnosis or even the alleviation of an immediate state of disease.

3. Medicine, in all its contacts, should be concerned with the whole man as a human personality, with the mind and the emotions as well as the heart and the lungs.

By 1935, when Winter's third term as Dean of the School came to an end, every one of his immediate ideals had been realized. The medical sciences were housed in a convenient and dignified group of buildings. The faculty included twenty-seven men of outstanding quality, of professorial rank. The endowment had been quadrupled since 1920. The first two basic principles enunciated in 1922, emphasis on research and on "the promotion of health as a positive ideal," had been measurably realized. The third, emphasis on "the whole man as a human personality" had made less progress; but this dream too began to bear fruit in the decade of the Forties.

Truly, this fifteen years was the Golden Age of the Yale School of Medicine; and Winter was its presiding genius. The task was not easy. There was inertia and, in some cases, active opposition. But his indomitable courage and his selfless absorption in the ideals which he visualized triumphed over obstacles. He raised this School from the rear of the procession to a place of leadership, and he opened new horizons for the entire discipline of medical education.

After fifteen years devoted with unswerving energy to administrative planning, readjustment to the life of a productive scholar must have presented real problems. It has always seemed to me specially characteristic of Winter's fibre and resilience that this adjustment was made with promptness and success. He turned wholeheartedly to the laboratory and initiated a series of anatomical studies of vascular disease which led to an important volume on the *Biology of Arteriosclerosis.* The approach which he developed is still—in the hands of his colleagues—yielding results on the relation of acute to chronic vascular disease which promise to be of fundamental importance in dealing with the control of disorders of later life which are becoming more and more central in the public health program of the present day.

During the Second World War—as during the First—Winternitz led in the Army research on war gases. He was largely instrumental in securing appropriations for the Medical Research Laboratory at Edgewood Arsenal, and in organizing the Coordination Center for information in regard to insect and rodent control, which is proving highly fruitful at the present time.

He has passed on with undiminished energy and enthusiasm to new duties as Acting Chairman of the Division of Medical Sciences of the

National Research Council. At New Haven, we shall sorely miss his vivid energy, his explosive and illuminating comments, his questioning spirit, and his basic loyalties. But he will not be forgotten by those who love Yale and by those who believe in a great future for the medical sciences and for medical education.

What I had the privilege of saying to Winter fifteen years ago, I say once more: "Times change, and men, and manners. But visions in the end prevail; and those visions, which you have built into the very fabric of this University and into the very fiber of its collective mind, will come to their realization and will beget new and wider visions in the days to come."

COMMENTS UPON THE MEDICAL CURRICULUM

Excerpts from Reports to the President and Fellows of Yale University

MILTON C. WINTERNITZ

Dean of the School of Medicine, 1920-1935

1921-22. At the beginning of the last academic year a radical change was made in the curriculum. It has been a time-honored custom to assign students who are just commencing the study of clinical disease at the beginning of their third year, to the out-patient clinic where they obtained a fleeting glance of a multitude of individuals in many stages of health and disease, while the Seniors in their fourth year were admitted to the wards to study individually and at length disease in bedridden patients. For many reasons it has been decided to reverse this order, and the result of the change has been most gratifying.

The criticism has been justly made of medical education that its conception of disease is too restricted and that only after graduation does the physician acquire a knowledge of the life history of disease. Too often in his school years the student's attention is monopolized by hospital cases which manifest only a stage in the history of the patient, and the larger field as it may present itself in the ambulatory clinic frequently is utilized only for minor courses of physical diagnosis, except in certain specialties. By reversing this old order it is felt that a step forward has been taken.

1922-23. The benefit to the student is most obvious in the type of thesis presented for graduation. The requirement of a thesis has long been considered of importance by the Faculty, but heretofore the student has been handicapped by the fact that, although a thesis was demanded, adequate time was not allowed for its preparation. It is not expected that every thesis will be of paramount importance; indeed, there is no known method for selecting students which will assure any large number of promising investigators at the end of the medical course. The most that can be done is to put the student in the proper atmosphere, to provide opportunity, and to place a premium on research. Even then a school should be considered fortunate if the various classes ultimately produce a small number of investigators. The thesis aids in this desired result, and with the time now available for its preparation, extending over a period of three years, it is gratifying to know that a considerable number of these studies are worthy of publication.

While research is an aim of the School, it is not the only one. The primary responsibility is to give the student a thorough foundation in medicine; but, in order to develop his initiative and judgment in analyzing the problems of health and disease, emphasis must be placed on original investigation.

1924-25. Specifically, the plan under consideration is an abolition of the present year system with its resultant division of the student body into classes. The student would be allowed to select the sequence of his studies from the courses offered in the School; the number and arrangement of courses to be completed in any period of time would be largely a matter of choice and ability on the part of the student. Obviously, admission to a course would depend upon the student's preparation for that particular discipline and would be subject to the approval of the responsible instructor . . .

These changes may seem radical, but after all they are in accord with adopted systems of graduate education in which Medicine should be included. There must, of course, be some check on the student's accomplishments. Group examinations, as well as the graduating thesis, would serve this purpose. For the convenience of the faculty such examinations may be given at fixed times, but within reasonable limits the student may determine when he will present himself for such a test . . . It is hoped that by the elimination of the class system, the pupil who acquires knowledge less rapidly will be less reluctant to spend more time in preparation, while the more brilliant scholar will be more willing to spend longer periods in investigation and specialization.

1930-31. In our approach to problems of education it is necessary to determine what we mean by the term "education". How to learn and how to teach others what you have learned are two different problems. Universities should be interested primarily in providing opportunity for learning and not in giving instruction. Teaching need enter only in so far as there is failure to learn . . . Every effort must be made not to stifle the opportunities for learning by building up a great machine for teaching. . . . In short, a university should protect learning against the inroads of teaching and methodology of all sorts.

1931-32. Such a liberal system of education places much responsibility upon the students, who in general have responded satisfactorily to the opportunities offered them. The burden that falls upon the instructional staff, it must be understood, is greatly augmented through this system, because didactic lectures are in large part replaced by small group conferences, and because of the large number of elective opportunities. The conferences, though interesting, are time-consuming both in conduct and in preparation. The electives, the majority of which are only open to a few students at a time, are demanding upon the energy of the instructor because of the individual attention which he is inclined to give to the more responsive students. Most students take electives, it is true, with the result that many of those who do are students of average ability. These are likely to get less attention from instructors than the few who are brilliant. Students of average ability, therefore, may lack guidance to a certain extent and may have difficulties for this reason until they become accustomed to relying upon their own initiative, as most of them ultimately do. . . .

To give the student further opportunity, as well as to take advantage of the extraordinary facilities that are offered in the School through associ-

ation with staff members, it has been the policy to keep every department actively at work throughout the calendar year. Formal instruction is not available through the vacation periods, but laboratories and clinics remain active, the former geared perhaps to a lower level of activity. For the student these vacation periods often yield the greatest harvest of his training period. His associations with his instructors are more intimate, and freedom from even a relatively small amount of required work permits the use of interest and intelligence in a way that is well-nigh impossible at other periods, when the time of student and faculty member is so likely to be absorbed by regular duties. . . .

The attitude of students toward the School after graduation has become more and more one of interest and loyalty. The comment most frequently made by returning graduates is that their preparation has fitted them to meet the problems of medicine more logically and intelligently than their colleagues from many other schools, even though the latter at the beginning of their professional careers often seem to possess greater factual knowledge. This indicates that the objectives of the educational policy at Yale, namely, to enable the student to solve his problems by the use of intelligence rather than memory, are being approached.

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